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02447121.1

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For the President of the European Patent Office

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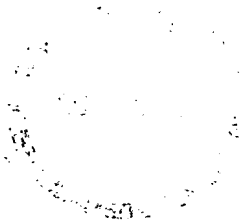

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
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Method for the diagnosis and differential diagnosis of neurological diseases

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METHOD FOR THE DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF NEUROLOGICAL DISEASES

FIELD OF THE INVENTION

The present invention relates to the diagnosis and differential diagnosis of neurological diseases. More specifically, the present invention provides new biomarkers for the screening, diagnosis or prognosis of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression. The present invention further provides new biomarkers for the differential diagnosis of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

BACKGROUND ART

Alzheimer's disease (AD) is the most common type of senile dementia and is believed to be responsible for 70% of all cases of dementia. The incidence of AD increases with age, affecting 1 out of 10 persons older than age 65 and nearly 1 out of 2 persons older than age 85. The disease is characterized by cognitive disorders associated with a loss of memory and orientation, and deterioration of the intellectual capacity of the patients (Duyckaerts et al. 1999). Two major types of histological lesions are observed in AD brains, in association with neuronal loss (Felician and Sandson, 1999): (i) at the intracellular level, the neuronal cytoskeleton in AD patients is progressively disrupted and replaced by neurofibrillary tangles (NFTs) composed of paired helical filaments (PHF); (ii) at the extracellular level, senile plaques are formed by deposits of fibrillary β-amyloid (Aβ). In view of the large expenses for health care systems that must provide institutional and ancillary care for the AD patients, the impact of AD on society and on national economies is enormous.

At present, AD is diagnosed by physical examination, by a detailed neurological and psychiatric examination, by a mental status assessment and by formal cognitive tests (Zagaria, 2001). For patients with recent-onset dementia, focal neurologic signs or atypical symptoms, neuroimaging studies such as CT scanning or magnetic resonance imaging (MRI) can help in the diagnostic evaluation (Daly, 1999). Postmortem identification of senile plaques and NFT, however, remains the only generally accepted way to confirm the diagnosis of AD.

Vascular dementia (VAD) is the second most common cause of dementia, accounting for about 20% of all cases by itself, and up to another 20% in combination with AD. VAD usually affects people between the ages of 60 and 75 and is slightly more common in men than women. VAD is a term for dementia associated with problems in the circulation of the blood to the brain (cerebrovascular disease). VAD is not a single disease but a group of syndromes relating to different vascular mechanisms. When blood vessels in the brain burst (cerebral hemorrhage), when arteries are blocked by plaque formation or clots (thrombosis or embolism), or when there is insufficient blood flow to parts of the brain (ischaemia), brain tissue will die. This is often called a stroke. It is thought that about a fifth of the people who have strokes will develop problems involving their mental abilities including dementia. Based on the current accepted criteria, VAD can still be very difficult to distinguish from other forms of dementia because of the great overlap with AD (Alagiakrishnan and Masaki, 2001).

Diagnosis of VAD is done by physical examination, by brain imaging tests such as computerized tomography (CT) scan or magnetic resonance imaging (MRI), and by psychological tests (Alagiakrishnan and Masaki, 2001). However, as in the case with AD, a diagnosis of definite VAD can only be made by examining the brain at autopsy. Sometimes VAD is difficult to distinguish from AD and/or depression (Alagiakrishnan and Masaki, 2001). As some vascular problems can be treated in VAD, an early and correct diagnosis of VAD is crucial.

Frontotemporal dementia (FTD) is a degenerative condition of the front (anterior) part of the brain. It occurs predominantly after age 40 and usually before age 65, with equal incidence in men and women. FTD is characterized by cerebral atrophy in the

frontal and anterior temporal lobes of the brain, while AD affects the hippocampal, posterior temporal and parietal regions. The NFT and senile plaques present in the brains of AD are absent. FTD is marked by dramatic changes in personality, behavior and some thought processes. Changes in personal and social conduct occur in the early stages of the disease, including loss of inhibition, apathy, social withdrawal, hyperorality (mouthing of objects), and ritualistic compulsive behaviors. These symptoms may lead to misdiagnosis as a psychological or emotionally based problem. FTD progresses to immobility and loss of speech and expression. FTD may account for about 8-10% of the dementia cases (Kirshner, 2001).

FTD is currently diagnosed with brain scans or imaging and clinical examination. Computed tomography (CT) scan and magnetic resonance imaging (MRI) reveal cerebral atrophy in the frontotemporal regions. Functional imaging techniques, particularly single photon emission computed tomography (SPECT) and positron emission tomography (PET), detect focal lobar hypometabolism or perfusion with great sensitivity. FTD is further diagnosed by neuropsychological testing and evaluation by a speech/language pathologist with standardized language batteries (Kirshner, 2001). The current treatment of AD patients, acetylcholinesterase-inhibitors, is not effective in FTD patients (Moghul and Wilkinson, 2001). Therefore, a correct differential diagnosis between AD and FTD is crucial.

Dementia with Lewy bodies (DLB) is an illness that presents with progressive dementia or psychosis. Parkinsonian signs, which may be absent or mild at the onset, eventually become common and rigidity is usually severe. Lewy bodies are found profusely in the brainstem, basal forebrain, hypothalamic nuclei and neocortex. DLB is characterized by the relative absence of tangles and hyperphosphorylated tau in the brain. Autopsy studies suggest that DLB accounts for 10 to 20% of dementias (Crystal, 2001). Parkinson's disease (PD) is a type of Lewy Body disease occurring in the middle or late life, with very gradual progression and a prolonged course. It can be considered as an example of neuronal system disease, involving mainly the nigrostriatal dopaminergic system. Dementia with Lewy bodies is defined as a special form of dementia requiring differential patient management (Lebert et al., 1998; McKeith et al., 1999).

Dementia with Lewy bodies is clinically very difficult to differentiate from Alzheimer's disease (McKeith et al., 1996; Ballard et al., 1998). Most patients (more than 75%) are neuropathologically defined as Alzheimer's disease patients while it is estimated that 15 to 25 % of the clinically diagnosed Alzheimer's disease patients have dementia with Lewy bodies (Hooten and Lyketsos, 1998). As dementia with Lewy bodies is more susceptible to acetylcholinesterase treatment, differentiation of dementia with Lewy bodies from Alzheimer's disease is essential for optimization of treatment (Levy et al., 1994; Perry et al., 1994; Wilcock and Scott, 1994). In addition, neuroleptic drugs, prescribed for psychiatric symptoms, may have irreversible complications in DLB (McKeith, 2002). Therefore also a correct differential diagnosis of DLB is crucial.

A depressive disorder is an illness that involves the body, mood, and thoughts. It affects the way a person eats and sleeps, the way one feels about oneself and the way one thinks. The underlying pathophysiology of major depressive disorder (MDD) is not ill-defined. Clinical and preclinical trials suggest a disturbance in CNS serotonin activity as an important factor. In the US, lifetime incidence of MDD is 20% in women and 12% in men (Aronson, 2002).

Diagnosis of depression includes physical examination and a mental status examination. As depression can be treated, it is important to diagnose depression correctly and to clearly differentiate depression from dementia.

Most neurological conditions for which the patient seeks general medical care can be identified by a combination of different investigations. Only after accurate diagnosis an effective management and treatment of the disease is possible.

As indicated above, some techniques for diagnosis of neurological diseases in patients have been developed such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and nuclear magnetic resonance spectroscopy (NMRS), making it possible to study brain function and structure. Most neurological diseases, however, are still only diagnosed clinically. Clinical evaluation of neurological diseases, however, is complex, as the physician must rule out other problems or disorders that exhibit similar symptoms.

In view of the discovery of disease-modifying compounds, which are likely to have their maximal benefit in the early stages of disease and well before neurodegeneration is widespread, there is a great need for reliable early diagnosis of AD and other neurological diseases, and for an accurate differential diagnosis between neurological diseases. Biochemical diagnostic markers (biomarkers), which reflect the pathogenic processes in the brain, can add to the accuracy of this early and differential diagnosis. A number of candidate biomarkers for neurological diseases have been identified. Lütjohann et al. (2000), for example, noted a slight increase in 24S-hydroxycholesterol in plasma of AD and VAD patients compared to the level in healthy controls and depressed patients. Montine et al. (1998 and 2000) report on increased concentrations of prostaglandin E2 and F2-isoprostanes and decreased concentrations of 6-keto-PGF1α in AD patients. The light subtype of the neurofilament protein was increased in AD patients compared with controls (Sjogren et al. 2001). Several CSF proteins, analyzed by 2-dimensional electrophoresis, have been suggested as diagnostic markers for degenerative disorders. Examples are 14-3-3 brain protein, p130 and p131 as markers for Creutzfeldt-Jacobs disease (Zerr et al., 1996; Hsich et al., 1996), and the middle isoform of α-2 haptoglobulin for Alzheimer's disease and schizophrenia (Johnson et al., 1992). Levels of glutamine synthetase were significantly increased in CSF from patients with AD and to a lesser extent in patients with VAD (Tumani et al. 1999). CSF-phospho-tau levels were increased in AD patients compared with age-matched controls, while decreased in patients with FTD (Vanmechelen et al. 2000). Phospho-tau was also shown to be a good marker for the differential diagnosis of AD versus DLB and AD versus FTD (International patent application published under WO 02/03073). Combined measurements of β-amyloid and tau in CSF have become a valuable diagnostic tool during the recent years, predicting more than 80% of AD cases (Andreasen et al. 1999; 2001). However, in order to further increase the predictability of AD, especially early in the course of the disease, to improve the early diagnosis for other neurological diseases and to provide for the differential diagnosis between neurological diseases, there is a substantial need to find additional new, complementary disease markers.

SUMMARY OF THE INVENTION

The present invention provides a method for the screening, diagnosis and/or prognosis in a mammal of one or more neurological diseases, for identifying a mammal at risk of developing one or more neurological diseases or for monitoring the effect of therapy administered to a mammal having one or more neurological diseases.

More specifically, the present invention provides a method for the screening, diagnosis and/or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention provides a method for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention provides a method for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention also provides a method for the differential diagnosis in a mammal of different neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The method of the invention comprises the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins: α-1-antitrypsin, α-1-β glycoprotein, antithrombin III, Apo A-I, Apo A-IV, Apo E, Apo J, gelsolin, haptoglobin, hemopexin, Ig α-1 chain C region (heavy), kininogen, prostaglandin-H2 D-isomerase, transthyretin, vitamin D-binding protein, Zn-α-2-glycoprotein, or of an isoform thereof; and

(b) comparing the level of said at least one protein or protein isoform detected in step (a) with the level of said at least one protein or protein isoform in a control mammal or in a mammal suffering from another neurological disease; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, dementia with Lewy bodies, Frontotemporal dementia, Vascular dementia and/or depression.

The present invention further provides protein isoforms that are associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention also provides a method for the detection of the protein isoforms of the invention, associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention further provides antibodies that specifically recognize the protein isoforms of the invention.

The present invention further provides a kit for the screening, diagnosis and/or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present inventions also provides a kit for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention also provides a kit for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention further provides a kit for the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention further provides a kit for the detection of the protein isoforms of the invention associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention further provides a method of screening for agents that interact and/or modulate the expression or activity of a protein isoform of the invention, associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

FIGURE LEGENDS

Figure 1. Digital CSF 2D master map with annotated spots. All annotated spots show an altered protein isoform expression pattern between AD, FTD and/or control patients and were identified by MS sequencing. Spot numbers are indicated in Table 3.

Figure 2. Black dots on Western blots indicate the immunoreaction of Apo E with the anti Apo E M-012 antibody (INNOGENETICS N.V., Gent, Belgium). The original gel (12.5%, pI 4.5-5.5, 18 cm) was loaded with 300 µL AD1 CSF sample. The 2D SDS-PAGE and blotting were performed as described in the example section.

Figure 3. 2D-gel image of CSF samples obtained from patients with depression. Separation was done on a pH 4.4-5.5 IPG strip in the first dimension. Approximately 600 µl of CSF sample, from which human serum albumin and immunoglobulin G were depleted, was applied the IPG strip. The number labels on the 2D pattern indicate protein isoforms that were significantly increased or decreased compared to CSF samples isolated from AD patients. Spot numbers are indicated in Table 4.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for the screening, diagnosis and/or prognosis in a mammal of one or more neurological diseases among which Alzheimer's disease (AD), Frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), Vascular dementia (VAD) and/or depression (D), to a method for identifying a mammal at risk of developing on or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or to a method for monitoring the effect of a therapy administered to a mammal having one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression. The method of the invention comprising the following steps:

(a) detecting, in the mammal under examination, the level of at least one of the following proteins: α-1-antitrypsin, α-1-β glycoprotein, antithrombin III, Apolipoprotein A-I (Apo A-I), Apolipoprotein A-IV (Apo A-IV), Apolipoprotein E (Apo E), Apolipoprotein J (Apo J), gelsolin, haptoglobin, hemopexin, Ig α-1 chain C region (heavy), kininogen, prostaglandin-H2 D-isomerase, transthyretin, vitamin D-binding protein, Zn-α-2-glycoprotein, or of an isoform thereof; and

(b) comparing the level of said at least one protein or protein isoform detected in step (a) with the level of said at least one protein or protein isoform in a control mammal; and

(c) concluding form the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, an altered level of said at least one protein or protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease, dementia with Lewy bodies, Frontotemporal dementia, Vascular dementia and/or depression.

The present invention further relates to a method for the differential diagnosis in a mammal of different neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression. The method of the invention comprising the following steps:

(a) detecting, in the mammal under examination, the level of at least one of the following proteins: α-1-antitrypsin, α-1-β glycoprotein, antithrombin III, Apo A-I, Apo A-IV, Apo E, Apo J, gelsolin, haptoglobin, hemopexin, Ig α-1 chain C region (heavy), kininogen, prostaglandin-H2 D-isomerase, transthyretin, vitamin D-binding protein, Zn-α-2-glycoprotein, or of an isoform thereof; and

(b) comparing the level of said at least one protein or protein isoform detected in step (a) with the level of said at least one protein or protein isoform in a mammal suffering from another neurological disease; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention is based on the finding that, the levels of the above-indicated proteins are significantly altered in CSF samples obtained from AD patients, FTD patients, DLB patients, VAD patients and/or patients with depression compared to CSF samples obtained from control patients. The inventors further found that these protein profiles are differentially altered in CSF samples obtained from AD patients, FTD patients, DLB patients, VAD patients and/or patients with depression. The indication that the level of the above proteins differs between patients with AD, FTD, DLB, VAD, depression and/or control patients forms the basis for the development of a diagnostic test for the diagnosis and differential diagnosis of said neurological diseases in mammals.

More particularly, the present inventors were able to identify specific protein isoforms that are significantly altered in CSF samples obtained from AD patients, FTD patients, DLB patients, VAD patients and/or patients with depression compared to CSF samples from control patients. The inventors further found specific protein isoforms that are differentially altered in CSF samples obtained from AD patients, FTD patients, DLB patients, VAD patients and/or patients with depression.

A "protein isoform" refers to variants of a polypeptide that are encoded by the same gene, but that differ in their isoelectric point (pI) or molecular weight (MW), or both. Such isoforms can differ in their amino acid composition (e.g. as a result of alternative mRNA or premRNA processing, e.g. alternative splicing or limited proteolysis) and in addition, or in the alternative, may arise from differential post-

translational modification (e.g. glycosylation, acylation, phosphorylation) or can be metabolically altered (e.g. fragmented). In the present invention CSF from mammals with AD, FTD, DLB, VAD or depression was analyzed for quantitative and qualitative detection of one or more protein isoform. A protein isoform of which the level is altered in CSF from mammals with AD, FTD, DLB, VAD, depression or another neurological disease is also called a "neurological disease-associated protein isoform" or "NPI". The NPIs of the present invention are listed in Tables 3, 4, 5 and 6.

A NPI, thus, is a protein comprising a peptide sequence described for that protein which has a pI on 2D gel electrophoresis of about the value stated in Table 3, 4, 5 or 6 for that NPI (preferably within about 10%, more preferably within about 5%, still more preferably within about 1% of the stated value) and which has a MW on 2D gel electrophoresis of about the value stated in Table 3, 4, 5 or 6 for that NPI (preferably within about 10%, more preferably within about 5%, still more preferably within about 1% of the stated value).

Accordingly, the present invention provides a method for the screening, diagnosis and/or prognosis in a mammal of one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, a method for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or a method for monitoring the effect of a therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression. The method of the invention comprising the following steps:

(a) detecting, in the mammal under examination, the level of at least one of the following protein isoforms (Table 3; Table 4; Table 5; Table 6):
   - α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
   - α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
   - Antithrombin-III: NPI 4;
   - Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
   - Apo A-IV: NPI 8, NPI 9, NPI 10;
   - Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;

- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, dementia with Lewy bodies, Frontotemporal dementia, Vascular dementia and/or depression, an altered level of said at least one protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease, dementia with Lewy bodies, Frontotemporal dementia, Vascular dementia and/or depression.

The present invention further provides a method for the differential diagnosis in a mammal of one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or of depression. The method of the invention comprising the following steps:

(a) detecting, in the mammal under examination, the level of at least one of the following protein isoforms (Table 3; Table 4; Table 5; Table 6):
- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;

- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from another neurological disease; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The proteins and protein isoforms as indicated above thus present new biomarkers for use in the diagnosis of neurological diseases.

The mammal examined in the present invention may be a non-human mammal, such as (but not limited to) a cow, a pig, a sheep, a goat, a horse, a monkey, a rabbit, a hare, a dog, a cat, a mouse, a rat, an elk, a deer or a tiger. In a preferred embodiment, the mammal is a primate. In another preferred embodiment the mammal is a human, more preferably the mammal is a human adult.

The method of the present invention can also be used in animal models, for example, for use in drug screening. The animal model on which the method of the present invention can be applied can be any model of an animal in which the body control system is directed by CNS. The animal thus may belong to the Platyhelminthes,

Aschelminthes, Annelida, Arthropoda, Mollusca, Echinodermata, Acrania, Cyclostomata, Chondrichthyes, Osteichthyes, Amphibia, Reptilia, Aves and Mammalia. In a preferred embodiment, the animal in the animal model is a mouse, a rat, a monkey, a rabbit, a worm or a fly.

A "control mammal", as defined in the present invention is a mammal of the same species as the mammal under examination which is free from AD, FTD, DLB, VAD and depression. Preferably, the control mammal is free from any neurological disease. A mammalian species as used in the present invention refers to the lowest taxonomic classification used that differentiates between mammals that can actively reproduce with one another and produce fertile offspring. 'A mammal of the same species' as used in the present invention, therefore is a mammal that can actively reproduce with the mammal suspected to suffer a neurological disease. In a preferred embodiment, a reference level range for the control mammal can be determined for a certain NPI in a mammal free from AD, FTD, DLB, VAD and depression. The levels obtained in mammals suspected to suffer AD, FTD, DLB, VAD and/or depression can then be compared with the previously determined reference level range.

The term "level" or "levels", as used in the present invention, refers to the presence and/or amount of a protein or protein isoform. The level can be determined qualitatively or quantitatively. A "qualitative" change in the protein or protein isoform level refers to the appearance or disappearance of a protein spot that is respectively not detectable or is present in samples obtained from control mammals or from mammals suffering another neurological disease. A "quantitative" change in the level of a protein or protein isoform refers to a measurable increase or decrease in the protein or protein isoform level when compared to control mammals or to mammals suffering another neurological disease. For any given NPI, the level obtained upon analyzing a mammal suspected of suffering a certain neurological disease relative to the level obtained upon analyzing a control mammal or a mammal suffering another neurological disease will depend on the particular analytical protocol and detection technique that is used. Accordingly, those skilled in the art will understand that any laboratory, based on the present description, can establish a suitable reference range for any NPI in control mammals and/or in mammals suffering AD, FTD, DLB, VAD and/or depression according to the analytical protocol and detection technique in use.

An 'altered level of the protein or protein isoform' as used in the present invention refers to the appearance or disappearance of the protein or protein isoform under

examination (qualitative detection or QL; Tables 3 and 4) or to the increase or the decrease of the protein or protein isoform under examination (quantitative detection or QN; Tables 3 and 4) in mammals with a certain neurological disease relative to control mammals or relative to mammals suffering another neurological disease.

In the method of the present invention, at least one of the proteins or protein isoforms associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression (indicated in Tables 3, 4, 5 and/or 6) is detected. It is clear that also more than one of the above proteins or protein isoforms can be detected simultaneously. Detection of an appropriate combination of more than one biological marker will often increase the specificity and sensitivity of the method. Therefore, in a preferred embodiment, a combination of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 68, at least 71 or at least 77 protein isoforms is detected in the method of the invention. When multiple proteins or protein isoforms are detected this is also called a protein profile. The term "protein profile" refers to a group of specific proteins present in samples obtained from mammals with neurological diseases in which differences can be detected when compared to control mammals. A disease-specific protein profile is obtained by comparing the level of a variety of proteins in a sample taken from a control mammal to the levels found in samples taken from affected mammals. The proteins that comprise the profile may be unaltered, increased, decreased, present or absent with respect to the control mammal.

In any of the above methods, detection of at least one NPI may optionally be combined with detection of one or more additional known biomarkers for neurological diseases, including but not limited to amyloid β peptides, tau, phospho-tau, synuclein, Rab3a and neural thread protein.

"Diagnosis" as used in the present invention refers to diagnosis, prognosis, monitoring, selecting participants in clinical trials, and identifying patients most likely to respond to a particular therapeutic treatment. Treatment refers to therapy, prevention and prophylaxis. The method of the invention can also be used for

monitoring the effect of therapy administered to a mammal, also called therapeutic monitoring, and patient management. Changes in the level of the protein and/or protein isoform as identified above and associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, can also be used to evaluate the response of a mammal to drug treatment. In this way, also new treatment regimes can be developed by examining the level of the protein or protein isoform in a mammal. The method of the present invention can thus assist in monitoring a clinical study, for example, for evaluation of a certain therapy for AD, FTD, DLB, VAD and/or depression. In this case, a chemical compound is tested for its ability to normalize the level of a NPI in a mammal having AD, FTD, DLB, VAD and/or depression to levels found in control mammals. In a treated mammal, a chemical compound can be tested for its ability to maintain the NPI level at or near the level seen in control mammals.

The present invention further provides for methods for the differential diagnosis of neurological diseases. "Differential diagnosis of an individual suffering from a neurological disease versus an individual suffering from another neurological disease" as used in the present invention refers to the discrimination between said first neurological disease and other neurological diseases in this way that a certain neurological disease or a certain cause of neurological disease in an individual is associated with a certain neurological condition of said individual. The method of the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease, from Frontotemporal dementia, from dementia with Lewy bodies, from Vascular dementia and/or from depression. In a specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from Frontotemporal dementia (FTD). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from dementia with Lewy bodies (DLB). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from Vascular dementia (VAD). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from depression. In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from

Frontotemporal dementia (FTD) versus an individual suffering from dementia with Lewy bodies (DLB). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Frontotemporal dementia (FTD) versus an individual suffering from Vascular dementia (VAD) . In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Frontotemporal dementia (FTD) versus an individual suffering from depression. In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from dementia with Lewy bodies (DLB) versus an individual suffering from Vascular dementia (VAD). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from dementia with Lewy bodies (DLB) versus an individual suffering from depression. In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Vascular dementia (VAD) versus an individual suffering from depression.

Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and depression as well as other neurological diseases have been described in detail by Wilson et al. (1991) and McKeith et al. (1999).

Different groups of NPIs, each with a different behaviour (appearance, disappearance, increase or decrease) in the various neurological diseases, were isolated and identified (see Tables 3, 4, 5 and 6 and the example section). A first group comprises the NPIs that are decreased in mammals having AD as compared to control mammals (C>AD). This group includes NPI 11, NPI 16, NPI 24 and NPI 74. Accordingly, in one embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 11, NPI 16, NPI 24, NPI 74; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at

least one protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease.

A second group comprises the NPIs that are increased in mammals having AD as compared to control mammals (AD>C). This group includes NPI 1, NPI 73, NPI 76, NPI 77. Accordingly, in one embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least the following protein isoforms (Table 3; Table 6): NPI 1, NPI 73, NPI 76, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether of the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease.

A third group comprises the NPIs that are decreased in mammals having FTD as compared to control mammals (C>FTD). This group includes NPI 5, NPI 12, NPI 17, NPI 18, NPI 25 and NPI 74. Accordingly, in one embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 5, NPI 12, NPI 16, NPI 17, NPI 18, NPI 25, NPI 74; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Frontotemporal dementia, a decreased level of said at least one protein isoform being an indication of the mammal under examination suffering from Frontotemporal dementia.

A fourth group comprises the NPIs that are increased in mammals having FTD as compared to control mammals (FTD>C). This group includes NPI 4, NPI 6, NPI 8, NPI 9, NPI 10, NPI 11, NPI 19, NPI 20, NPI 22, NPI 23, NPI 28, NPI 73, NPI 76 and NPI 77. Accordingly, in one embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 4, NPI 6, NPI 8, NPI 9, NPI 10, NPI 11, NPI 19, NPI 20, NPI 22, NPI 23, NPI 28, NPI 73, NPI 76, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Frontotemporal dementia, an increased level of said at least one protein isoform being an indication of the mammal under examination suffering from Frontotemporal dementia.

A fifth group comprises the NPIs that are increased in mammals having AD as compared to mammals having FTD (AD>FTD). This group includes NPI 5, NPI 6, NPI 13, NPI 26 and NPI 77. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 3; Table 6): NPI 5, NPI 6, NPI 13, NPI 26, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in the mammal suffering from Frontotemporal dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

A sixth group comprises the NPIs that are decreased in mammals having AD as compared to mammals having FTD (FTD>AD). This group includes NPI 2, NPI 3,

NPI 7, NPI 8, NPI 9, NPI 11, NPI 14, NPI 15, NPI 16, NPI 20, NPI 21, NPI 27, NPI 28, NPI 29, NPI 30, NPI 37, NPI 69, NPI 70, NPI 71, NPI 73, NPI 74 and NPI 76. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 3; Table 5; Table 6): NPI 2, NPI 3, NPI 7, NPI 8, NPI 9, NPI 11, NPI 14, NPI 15, NPI 16, NPI 20, NPI 21, NPI 27, NPI 28, NPI 29, NPI 30, NPI 37, NPI 69, NPI 70, NPI 71, NPI 73, NPI 74, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from Frontotemporal dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

A seventh group comprises the NPIs that are decreased in mammals having AD as compared to mammals having depression (AD<D). This group includes NPI 5, NPI 6, NPI 7, NPI 12, NPI 23, NPI 31, NPI 32, NPI 33, NPI 34, NPI 35, NPI 36, NPI 37, NPI 38, NPI 40, NPI 41, NPI 42, NPI 43, NPI 44, NPI 45, NPI 46, NPI 47, NPI 48, NPI 51, NPI 52, NPI 53, NPI 54, NPI 55, NPI 56, NPI 58, NPI 59, NPI 60, NPI 61, NPI 63, NPI 68, NPI 69, NPI 70, NPI 72, NPI 73, NPI 75 and NPI 76. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 5; Table 6): NPI 5, NPI 6, NPI 7, NPI 12, NPI 23, NPI 31, NPI 32, NPI 33, NPI 34, NPI 35, NPI 36, NPI 37, NPI 38, NPI 40, NPI 41, NPI 42, NPI 43, NPI 44, NPI 45, NPI 46, NPI 47, NPI 48, NPI 51, NPI 52, NPI 53, NPI 54, NPI 55, NPI 56, NPI 58, NPI 59, NPI 60, NPI 61, NPI 63, NPI 68, NPI 69, NPI 70, NPI 72, NPI 73, NPI 75, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from depression; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from depression, being an indication of the mammal under examination suffering from Alzheimer's disease.

An eighth group comprises the NPIs that are increased in mammals having AD as compared to mammals having depression (AD>D). This group includes NPI 39, NPI 49, NPI 50, NPI 57, NPI 62, NPI 64, NPI 65, NPI 66, NPI 67, NPI 74 and NPI 77. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 6): NPI 39, NPI 49, NPI 50, NPI 57, NPI 62, NPI 64, NPI 65, NPI 66, NPI 67, NPI 74, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from depression; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in the mammal suffering from depression, being an indication of the mammal under examination suffering from Alzheimer's disease.

A nineth group comprises the NPIs that are decreased in mammals having AD as compared to mammals having VAD (VAD>AD). This group includes NPI 7, NPI 70, NPI 74 and NPI 76. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 5; Table 6): NPI 7, NPI 70, NPI 74, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from Vascular dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

A tenth group comprises the NPIs that are increased in mammals having AD as compared to mammals having VAD (AD>VAD). This group includes NPI 5, NPI 6, NPI 34, NPI 37, NPI 69, NPI 73 and NPI 77. Accordingly, in one embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 5; Table 6): NPI 5, NPI 6, NPI 34, NPI 37, NPI 69, NPI 73, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering from Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in the mammal suffering from Vascular dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

The level of one or more NPIs can be determined *in vitro* as well as *in vivo*. The method for the *in vitro* detection of the level of the NPI in a mammal comprises the steps of obtaining a sample from said mammal, determining the level of the NPI in said sample and comparing the obtained level in said sample with the level of said NPI in a sample taken from a control mammal or from a mammal suffering another neurological disease.

The term 'sample' refers to any source of biological material, for instance body fluids, brain extract, peripheral blood or any other sample comprising the NPI. In a preferred

embodiment, the level of the NPI is determined *in vitro* by analysis of the level of the NPI in a body fluid sample of the mammal. The term 'body fluid' refers to all fluids that are present in the mammalian body including but not limited to blood, lymph, urine and cerebrospinal fluid (CSF) comprising the NPI. The blood sample may include a plasma sample or a serum sample.

In a preferred embodiment of the present invention the level of the NPI is determined in a cerebrospinal fluid sample taken from the mammal. The term "cerebrospinal fluid" or "CSF" is intended to include whole cerebrospinal fluid or derivatives of fractions thereof well known to those skilled in the art. Thus, a cerebrospinal fluid sample can include various fractionated forms of cerebrospinal fluid or can include various diluents added to facilitate storage or processing in a particular assay. Such diluents are well known to those skilled in the art and include various buffers, preservatives and the like.

In accordance, the present invention relates to a method as described above, comprising the steps of:

(a) obtaining a cerebrospinal fluid sample from the mammal under examination; and

(b) detecting, in said cerebrospinal fluid sample, at least one of the following protein isoforms (Table 3; Table 4; Table 5; Table 6):

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;
- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;

- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68; and

(c) comparing the level of said at least one protein isoform detected in step (b) with the level of said at least one protein isoform in a CSF sample from a control mammal or from a mammal suffering from another neurological disease; and

(d) concluding from the comparison in step (c) whether the mammal under examination is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

Accordingly, in a specific embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 11, NPI 16, NPI 24, NPI 74; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least the following protein isoforms (Table 3; Table 6): NPI 1, NPI 73, NPI 76, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 5, NPI 12, NPI 17, NPI 18, NPI 25, NPI 74; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Frontotemporal dementia, a decreased level of said at least one protein isoform being an indication of the mammal under examination suffering from Frontotemporal dementia.

In another embodiment, the present invention relates to a method for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 4, NPI 6, NPI 8, NPI 9, NPI 10, NPI 11, NPI 19, NPI 20, NPI 22, NPI 23, NPI 28, NPI 73, NPI 76, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Frontotemporal dementia, an increased level of said at least one protein isoform being an indication of the mammal under examination suffering from Frontotemporal dementia.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 5, NPI 6, NPI 13, NPI 26, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in a CSF sample from a mammal suffering from Frontotemporal dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following proteins isoforms (Table 3; Table 5; Table 6): NPI 2, NPI 3, NPI 7, NPI 8, NPI 9, NPI 11, NPI 14, NPI 15, NPI 16, NPI 20, NPI 21, NPI 27, NPI 28, NPI 29, NPI 30, NPI 37, NPI 69, NPI 70, NPI 71, NPI 73, NPI 74, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from

Frontotemporal dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 5; Table 6): NPI 5, NPI 6, NPI 7, NPI 12, NPI 23, NPI 31, NPI 32, NPI 33, NPI 34, NPI 35, NPI 36, NPI 37, NPI 38, NPI 40, NPI 41, NPI 42, NPI 43, NPI 44, NPI 45, NPI 46, NPI 47, NPI 48, NPI 51, NPI 52, NPI 53, NPI 54, NPI 55, NPI 56, NPI 58, NPI 59, NPI 60, NPI 61, NPI 63, NPI 68, NPI 69, NPI 70, NPI 72, NPI 73, NPI 75, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from depression; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's diseas, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from depression, being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 6): NPI 39, NPI 49, NPI 50, NPI 57, NPI 62, NPI 64, NPI 65, NPI 66, NPI 67, NPI 74, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from depression;and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in the mammal suffering from depression, being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following proteins isoforms (Table 5; Table 6): NPI 7, NPI 70, NPI 74, NPI 76; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in the mammal suffering from Vascular dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

In another embodiment, the present invention relates to a method for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in a CSF sample taken from said mammal, the level of at least one of the following proteins isoforms (Table 5; Table 6): NPI 5, NPI 6, NPI 34, NPI 37, NPI 69, NPI 73, NPI 77; and

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a CSF sample from a mammal suffering from Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal under examination is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in the mammal suffering from Vascular dementia, being an indication of the mammal under examination suffering from Alzheimer's disease.

The present invention additionally provides a preparation comprising at least one of the following isolated protein isoforms associated with one or more neurological diseases among which, Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or a fragment thereof:

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;

- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;
- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68.

A NPI is isolated when it is present in a preparation that is substantially free of other proteins, i.e., a preparation in which less than 10% (particularly less than 5%, more particularly less than 1%) of the total protein present is contaminating protein(s). The NPI identified herein can be isolated and purified by standard methods including chromatography (e.g. ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other technique for the purification of proteins. Alternatively, once a recombinant nucleic acid that encodes the NPI is identified, the entire amino acid sequence of the NPI can be deduced from the nucleotide sequence of the gene-coding region contained in the recombinant nucleic acid. As a result, the protein can be synthesized by standard chemical methods known in the art.

The proteins or protein isoforms that are associated with one or more neurlogical diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with

Lewy bodies, Vascular dementia and/or depression may be qualitatively or quantitatively detected by any method known to those skilled in the art. They can be identified by their structure, by partial amino acid sequence determination, by functional assay, by enzyme assay, by various immunological methods, or by biochemical methods such as capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyper diffusion chromatography, two-dimensional liquid phase electrophoresis (2D-LPE; Davidsson et al. 1999) or by their migration pattern in gel electrophoreses. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used approach for separating proteins form complex mixtures (Patterson and Aebersold, 1995). It can be performed in one- or two-dimensional (2D) configuration. For less complicated protein preparation, one-dimensional SDS-PAGE is preferred over 2D gels, because it is simpler. However, SDS-PAGE often results in migrating or overlapping protein bands due to its limited resolving power. What appears to be a single band may actually be a mixture of different proteins. 2D gel electrophoresis incorporates isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second dimension, leading to a separation by charge an size (O'Farrell, 1975). 2D PAGE is a powerful technique for separating very complex protein preparations, resolving up to 10 000 proteins from mammalian tissues and other complex proteins (Klose and Kobalz, 1995; Celis et al., 1996; Yan et al., 1997). The proteins or protein isoforms of the present invention are identified by their isoelectric focusing point (pI) and their molecular weight (MW) in kilodaltons (kD). Accordingly, the present invention relates to a method as described above, characterized in that the level of protein or protein isoform is detected by isoelectric focussing followed by denaturing electrophoresis. Preferably, the step of denaturing electrophoresis uses sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The identification of the protein spots that were altered between the studied groups is shown in Table 2.

As indicated above, the level of protein or protein isoform can also be detected by an immunoassay. As used herein, an "immunoassay" is an assay that utilizes an antibody to specifically bind to the antigen (i.e. the protein or protein isoform). The immunoassay is thus characterized by detection of specific binding of the proteins or protein isoforms to antibodies. Immunoassays for detecting proteins or protein

isoforms may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (i.e. the protein or protein isoform) is directly measured. In competitive assays, the amount of analyte (i.e. the protein or protein isoform) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced (or competed away) from a capture agent (i.e. the antibody) by the analyte (i.e. the protein or protein isoform) present in the sample. In one competition assay, a known amount of the (exogenous) protein or protein isoform is added to the sample and the sample is then contacted with the antibody. The amount of added (exogenous) protein or protein isoform bound to the antibody is inversely proportional to the concentration of the protein or protein isoform in the sample before the exogenous protein or protein isoform is added. In one preferred "sandwich" assay, for example, the antibodies can be bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture the protein or protein isoform of interest present in the test sample. Other immunological methods include but are not limited to fluid or gel precipitation reactions, immunodiffusion (single or double), agglutination assays, immunoelectrophoresis, radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), Western blots, liposome immunoassays (LIA; Monroe et al., 1986), complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays or immunoPCR. An overview of different immunoassays is given in Wild D. (2001) and Ghindilis et al. (2002).

In a preferred embodiment, the level of the protein or protein isoform is determined by an immunoassay comprising at least the following steps:

(a) contacting the protein or protein isoform with an antibody that specifically recognizes the protein or protein isoform, under conditions suitable for producing an antigen-antibody complex; and

(b) detecting the immunological binding that has occurred between the antibody and the protein or protein isoform.

In another embodiment, the protein or protein isoform can be detected by a sandwich ELISA comprising the following steps:

(a) bringing said protein or protein isoform into contact with an antibody (primary antibody or capturing antibody) recognizing said protein or protein isoform, under conditions being suitable for producing an antigen-antibody complex;

(b) bringing the complex formed between said protein or protein isoform and said primary antibody into contact with another antibody (secondary antibody or detector antibody) specifically recognizing said protein or protein isoform, under conditions being suitable for producing an antigen-antibody complex;

(c) bringing the antigen-antibody complex into contact with a marker either for specific tagging or coupling with said secondary antibody, with said marker being any possible marker known to the person skilled in the art;

(d) possibly also, for standardization purposes, bringing the antibodies in contact with a purified protein or protein isoform reactive with both antibodies.

Advantageously, the secondary antibody itself carries a marker or a group for direct or indirect coupling with a marker.

The term "specifically recognizing", "specifically binding with", "specifically reacting with" or "specifically forming an immunological reaction with" refers to a binding reaction by the antibody to the protein or protein isoform which is determinative of the presence of the protein or protein isoform in the sample in the presence of a heterogeneous population of other proteins, other protein isoforms and/or other biologics. Thus, under the designated immunassay conditions, the specified antibody preferentially binds to a particular protein or protein isoform while binding to other proteins or protein isoforms does not occur in significant amounts.

Any antibody that recognizes the protein or protein isoform under examination can be used in the above method. Examples of antibodies known in the art that can be used in the immunoassay of the invention are listed in Table 7. These antibodies are known to bind the protein of which the NPI of the invention is itself a family member.

The present invention also relates to an antibody capable of specifically recognizing one of the following protein isoforms associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression:

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;

- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68.

As used herein, an "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu , alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. The basic immunoglobulin (antibody) structural unit is known to comprise a tetramer or dimer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having on "light" (about 25 kD) and on "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids, primarily responsible for antigen recognition. The terms "variable light chain (V_L)" and "variable heavy chain (V_H)" refer to these variable regions of the light and heavy chains respectively.

Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single variable fragments (ssFv), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic antibodies and epitope-binding fragments of any of the above, provided that

they retain the original binding properties. Also mini-antibodies and multivalent antibodies such as diabodies, triabodies, tetravalent antibodies and peptabodies can be used in a method of the invention. The preparation and use of these fragments and multivalent antibodies has been described extensively in International Patent Application WO 98/29442. The immunoglobulin molecules of the invention can be of any class (i.e. IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

The NPI or a fragment or derivative thereof can be use as an immunogen to generate the antibodies of the invention which specifically bind such an immunogen. Various host animals can be immunized by injection with the native or a synthetic version of the NPI or the fragment or derivative of the NPI, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to enhance the immunological response, depending on the host species, including but not limited to complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, or an adjuvant such as BCG (bacille Calmett-Gurein) or corynebacterium parvum. For the preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used, including but not limited to the hybridoma technique developed by Kohler and Milstein (1975), the human B-cell hybridoma technique (Kozbor et al., 1983) or the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985). Screening for the desired antibody can be done by techniques known in the art such as ELISA. For selection of an antibody that specifically binds a first NPI but which does not specifically bind to a second NPI, one can select on the basis of positive binding to the first NPI and the lack of binding to the second NPI. Thus, in a particular embodiment, the present invention provides an antibody that binds with greater affinity (particularly at least 2-fold, more particularly at least 5-fold, still more particularly at least 10-fold greater affinity) to a first NPI than to a second NPI. In another preferred embodiment, the present invention provides an antibody that binds with greater affinity (particularly at least 2-fold, more particularly at least 5-fold, still more particularly at least 10-fold greater affinity) to a first NPI than to a second NPI of the same protein. These antibodies are also called anti-NPI antibodies.

While various antibody fragments are defined in terms of enzymatic digestion of an intact antibody with papain, pepsin or other proteases, one of skill will appreciate that

such antibody fragments as well as full size antibodies may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibodies and antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. The humanized versions of the mouse monoclonal antibodies are also made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal antibodies used in the method of the invention may be human monoclonal antibodies. The term 'humanized antibody' means that at least a portion of the framework regions of an immunoglobulin is derived from human immunoglobulin sequences.

The antibodies used in the method of the present invention may be labeled by an appropriate label. The particular label or detectable group used in the assay is not a critical aspect of the invention, so long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well developed in the filed of immunoassays and, in general, most any label useful in such methods can be applied to the method of the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include but are not limited to magnetic beads (e.g. Dynabeads^{TM}), fluorescent dyes (e.g. fluorescein isothiocyanate, texas red, rhodamine), radiolables (e.g. ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g. horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold, colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

The label may be coupled directly or indirectly to the desired component or the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, the ease of conjugation with the compound, stability requirements, the available instrumentation and disposal provisions. Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g. biotin) is covalently bound to the antibody. The ligand then binds to an anti-ligand (e.g. streptavidin) molecule, which is

either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, an haptenic or antigenic compound can be used in combination with an antibody. The antibodies can also be conjugated directly to signal generating compounds, for example., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umberlliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophtlialazinediones, for example, luminol. A review of other labeling or signal producing system is available in US patent No. 4,391,904.

Means for detecting labels are well known in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorophore with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of a photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzyme labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

The method for the *in vivo* detection of the level of a protein or a protein isoform in a mammal comprises the steps of determining the level of said protein or protein isoform in said mammal and comparing it with the level of said protein or protein isoform in a control mammal or in a mammal suffering another neurological disease.

The level of said protein or protein isoform detected in the mammal under examination can also be compared with a previously defined level range characteristic for control mammals, or for mammals with FTD, DLB, VAD and/or depression. In an embodiment of the invention, the level of protein or protein isoform can be determined by *in vivo* imaging. The level of protein or protein isoform can be determined *in situ* by non-invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandrock et al. (1996), Mariani et al. (1997). These *in vivo* imaging methods may allow the localization and quantification of the protein or protein isoform, for example, by use of labeled antibodies (as described above) specifically recognizing said protein or protein isoform.

The invention also provides diagnostic kits comprising an anti-NPI antibody. The invention thus provides a diagnostic kit for the screening, diagnosis and/or prognosis in a mammal of one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, characterized that said kit comprises an anti-NPI antibody. The present invention thus also provides a diagnostic kit for the differential diagnosis in a mammal of different neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, characterized that said kit comprises an anti-NPI antibody. A preferred kit for carrying out the method of the invention comprises:

- an antibody (primary antibody) which forms an immunological complex with the protein or protein isoform to be detected;
- a monoclonal antibody (secondary antibody) which specifically recognizes the protein or protein isoform to be detected;
- a marker either for specific tagging or coupling with said secondary antibody;

- appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the protein or protein isoform, between the secondary antibody and the primary antibody-protein or -protein isoform complex and/or between the bound secondary antibody and the marker;
- possibly, for standardization purposes, a purified protein or protein isoform.

As it is known that the occurrence of some neurological diseases in a person is more prevalent at a certain age, age related kits can be prepared comprising antibodies that recognize specific proteins or protein isoforms that are associated with one or more neurlogical diseases that are more prevalent at that specific age.

In accordance, the present invention provides the antibody or the kit as defined above, for use in the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention also provides the antibody or the kit as defined above for use in the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

Also included in the present invention is the use of the antibody as defined above for the preparation of a kit for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

Also included in the present invention is the use of the antibody as defined above for the preparation of a kit for the differential diagnosis in a mammal of Alzheimer's

disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

The present invention also provide methods of screening for agents that interact with and/or modulate (have a stimulatory or inhibitory effect on) the expression or activity of a protein or protein isoform associated with one or more neurological diseases among which Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, said method comprising:

(a) contacting said protein or protein isoform or a portion of said protein or protein isoform with said agent; and

(b) determining whether or not said agent interacts with and/or modulates the expression or activity of said protein or protein isoform or said portion of the protein or protein isoform.

Candidate agents or test agents include, but are not limited to nucleic acids (DNA or RNA), carbohydrates, lipids, proteins, peptides, small molecules and other drugs. Agents can be obtained using any of the numerous suitable approaches in combinatorial library methods known in the art, including: biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method and synthetic library methods using affinity chromatography selection. Library compounds may be presented in solution, on beads, chips, bacteria, spores, plasmids or phage.

In one embodiment, a protein or protein isoform is identified in a cell-based assay system. In accordance with this embodiment, cells expressing the protein or protein isoform or a fragment thereof are contacted with the candidate agent or a control compound and the ability of the candidate agent to interact with the protein or protein isoform or to modify the biological behaviour of the cell is measured. The cell can be of prokaryotic origin (e.g. *E. coli*) or of eukaryotic origin (e.g. yeast or mammalian). The protein or protein isoform or the candidate agent can be labeled (described above), to enable detection of an interaction between the protein or protein isoform and the candidate agent. Interaction can then be detected by flow cytometry, by scintillation assay, by immunoprecipitation, by Western blot analysis, by its ability to modify or by other means.

In another embodiment, agents that interact with a protein or protein isoform are identified in a cell-free assay system. In accordance with this embodiment, a native or

recombinant protein or protein isoform or a fragment thereof is contacted with the candidate agent or a control compound and the ability of the candidate agent to interact with the protein or protein isoform is determined. Preferably, the protein or protein isoform or fragment thereof is first immobilized by, for example, contacting the protein or protein isoform the fragment thereof with an immobilized antibody that specifically recognizes said protein or protein isoform or said fragment thereof, or by contacting the protein or protein isoform or the fragment thereof with a surface designed to bind proteins.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of a protein or protein isoform or which is responsible for the post- translational modification of a protein or protein isoform. In a primary screen, a plurality (e.g., a library) of compounds are contacted with cells that naturally or recombinantly express: (i) a protein or protein isoform or a biologically active fragment thereof; and (ii) a protein that is responsible for processing of the protein or protein isoform or the fragment thereof in order to identify compounds that modulate the production, degradation, or post- translational modification of the protein or protein isoform. If desired, compounds identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific protein or protein isoform of interest. The ability of the candidate compound to modulate the production, degradation or post-translational modification of a protein or protein isoform can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and Western blot analysis.

In another embodiment, agents that competitively interact with (i.e., bind to) a protein or protein isoform or a fragment thereof are identified in a competitive binding assay. In accordance with this embodiment, cells expressing a protein or protein isoform or a fragment thereof are contacted with a candidate compound and a compound known to interact with the protein or protein isoform or the fragment thereof. The ability of the candidate compound to competitively interact with the protein or protein isoform or the fragment thereof is then determined.

Alternatively, agents that competitively interact with (i.e., bind to) a protein or protein isoform or a fragment thereof are identified in a cell-free assay system by contacting a

protein or protein isoform or a fragment thereof with a candidate compound and a compound known to interact with the protein or protein isoform or the fragment thereof. As stated above, the ability of the candidate compound to interact with a protein or protein isoform or a fragment thereof can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (e.g., a library) of candidate compounds.

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression of a protein or protein isoform are identified by contacting cells (e.g., cells of prokaryotic origin or of eukaryotic origin) expressing the protein or protein isoform with a candidate compound or a control compound (e.g., phosphate buffered saline (PBS)) and determining the expression of the protein or protein isoform or mRNA encoding the protein or protein isoform. The level of expression of a selected protein or protein isoform or mRNA encoding the protein or protein isoform in the presence of the candidate compound is compared to the level of expression of the protein or protein isoform, or mRNA encoding the protein or protein isoform in the absence of the candidate compound (e.g., in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of the protein or protein isoform, based on this comparison. For example, when expression of the protein or protein isoform or mRNA encoding the protein or protein isoform is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of expression of the protein or protein isoform or mRNA encoding the protein or protein isoform. Alternatively, when expression of the protein or protein isoform or mRNA encoding the protein or protein isoform is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of the protein or protein isoform or mRNA encoding the protein or protein isoform. The level of expression of a protein or protein isoform or the mRNA that encodes it can be determined by methods known to those of skill in the art based on the present description. For example, mRNA expression can be assessed by Northern blot analysis or RT- PCR, and protein levels can be assessed by Western blot analysis.

In another embodiment, agents that modulate the activity of a protein or protein isoform are identified by contacting a preparation containing the protein or protein isoform, or a cells (e.g., prokaryotic or eukaryotic cells) expressing the protein or protein isoform with a test compound or a control compound and determining the

protein or protein isoform. The activity of a protein or protein isoform can be assessed by different methods. The induction of a cellular signal transduction pathway of the protein or protein isoform (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.) can be detected. In other cases the catalytic or enzymatic activity of the target on a suitable substrate can be detected. The induction of a reporter gene (e.g., a regulatory element that is responsive to a protein or protein isoform and is operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase) can be measured. A cellular response, for example, cellular differentiation, or cell proliferation, as the case may be, can be detected. Based on the present description, techniques known to those of skill in the art can be used for and detecting these activities (see, e.g., U.S. Patent No. 5, 401,639). The candidate agent can then be identified as a modulator of the activity of a protein or protein isoform by comparing the effects of the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression, activity or both the expression and activity of a protein or protein isoform are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represents a model of Alzheimer's disease (for example: animals that express human familial Alzheimer's disease (FAD) amyloid precursor protein (APP), animals that overexpress human wild-type APP, animals that overexpress β-amyloid $_{(1\text{-}42)}$ (βA), animals that express FAD presenillin-1 (PS-1)), or a model for another neurological disease such as FTD, DLB, VAD or depression. In accordance with this embodiment, the test compound or a control compound is administered (e.g., orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of the protein or protein isoform is determined. Changes in the expression of a protein or protein isoform can be assessed by any suitable method described above, based on the present description.

WO 01/75454 enumerates other techniques and scientific publications describing suitable assays for detecting or quantifying enzymatic, modulating and/or binding activities of a protein or protein isoform or a fragment thereof. Each such reference is hereby incorporated in its entirety.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or group of integers or steps.

The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these examples are illustrative and can not be construed as to restrict the invention in any way.

EXAMPLES

Example 1: Materials and methods

1.1 Patients and CSF samples

The study was performed on CSF samples obtained from the Dept. of Clinical Neuroscience, Sahlgren's University Hospital, Mölndal, Sweden. An overview of all the CSF samples that were analyzed is given in Table 1. The neurological diseases from which the patients (from whom the CSF samples were taken) were suffering, is also indicated in Table 1 (diagnosis). The clinical criteria used for the diagnosis were based upon well-accepted standards. For the diagnosis of Alzheimer's disease, the NINCDS-ADRDA criteria (McKhann et al., 1984) were used. For the diagnosis of dementia with Lewy bodies, the criteria according to McKeith (McKeith et al., 1996) were used. For the diagnosis of Frontotemporal dementia, the Lund-Manchester guidelines (The Lund and Manchester Groups, 1994) were used. Vascular dementia was diagnosed according to the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neuroscience criteria (Roman et al., 1993). Diagnosis of depression was made according the DSM-IV criteria and the ICD-10 criteria (DSM-IV and International Classification of Diseases, 10th Revision). Table 1 also shows the level of tau, β-amyloid$_{(1-42)}$ (Ab42) and the albumin ratios present in the CSF samples. Data about the apoE alleles, sex, age and MMSE of the patients are also given.

1.2 Sample preparation

CSF samples were precipitated with 2 volumes equivalent of ice-cold acetone during two hours incubation at -20 °C. The protein pellets were collected by centrifugation and resolubilized in rehydratation buffer containing 8 M urea, 2% w/v CHAPS, 2% w/v IPG-buffer pH 4.5-5.5 and 1.8% DTT to yield the desired protein amount in a volume that was loaded on the Immobiline DryStrips pH 4.5-5.5. In-gel rehydratation was performed at room temperature over a period of 18 hours. Each CSF sample was run and analysed in duplicate.

1.3 IEF and 2D-PAGE

The separation of the proteins by their isoelectric point (first dimension) was performed at 115000 Vh (for 28 h at 18°C) using a Mutiphor II with a Pharmacia LKB Multidrive XL power supply (Amersham Pharmacia Biotech, Uppsala, Sweden) on Immobilized pH Gradients (IPG) prepared as described above. The theoretical pI values for all protein spots were approximately read out from drawing supplied with IPG narrow area strips. To separate proteins by molecular weight, ISO-DALT: 2D-PAGE system (Amersham Pharmacia Biotech, Uppsala) was used which allowed us to run ten gels simultaneously. Prior to staining, gels were fixed in a 10% methanol - 7% acetic acid solution during the 30 min. The spots were visualized with the fluorescent dye 'SYPRO Ruby' (Bio-Rad Laboratories, Hercules, CA, US). After overnight incubation in the staining solution gels were destained in 10% methanol - 7% acetic acid and digitized. The images were processed by the PDQuest 2D Gel Analysis Software suite (Bio-Rad Laboratories, Hercules, CA, US). The protein spots from different gels were matched and their spot volume was determined.

1.4 Protein identification

Selected protein spots were digested with trypsine *in situ*. The eluted peptides were sequenced by electrospray mass spectrometry. NanoLC-ESI-TOF-MS and tandem MS with column switching was used in the instrumental set up as already described in Raymackers et al. 2000. Briefly, the sample was injected on a Pep-Map C18 precolumn 0.3 mm × 1 mm (LC Packings, San Fransisco, CA, USA) followed by reverse elution on the nano-PepMap column 0.075 mm × 150 mm (LC Packings). The peptides were eluted from the nano-column by a gradient of acetonitrile / 0.1 % formic acid in water / 0.1 % formic acid at a flow rate of 230 nl/min. The column was directly coupled to the Q-TOF (Micromass, Wytenshawe, UK). The mass spectrometer was controled by the Masslynx 3.4 software (Micromass) directing automatic MS to tandem MS switching. The generated MS/MS spectra were automatically searched against human databases. MS/MS spectra that remained

uninterpreted after this search were sequenced manually and screened on protein and DNA databases.

1.5 Antibodies and Western blot analysis

For the determination of the total number of spots related to a particular protein, the established immunoblot method was used. Briefly, 300 µL of CSF sample were loaded on an IPG strip (Amersham Pharmacia Biotech, Uppsala, Sweden) and IEF was performed (for 7cm strip 7474 Vh or for 18 cm strip 71275 Vh). Samples separated by SDS-PAGE [12.5 or 4 – 20 % (w/v) gel] were electroblotted on nitrocellulose or PVDF membranes for immunological detection. Transfer was performed in Tris-Gly buffer (25 mM Tris, 192 mM glycine and 15 % methanol) at a current of 1mA/cm^2 of gel. The SuperSignal West Dura Extended Duration Substrate System (PIERCE, Rockford, US) was used for detection. The antibodies used in the Western blot assay are listed in Table 7.

1.6 Statistics

Statistical analysis was performed per matched protein spot using log transformation on the data. As in different cases quantitative data were not available, distinction was made between a qualitative and a quantitative analysis. For the quantitative analysis only data points were included in the analysis for which a numerical value was obtained. To test whether the quantity of a protein isoform was different between diagnostic groups pair-wise t test was performed. For the qualitative analysis, the number of patients for whom a quantitative value was obtained in both replicates was compared between diagnostic groups with the Fisher's exact test. This analysis reveals spots that occur more frequently in a given diagnostic group.

Example 2: Comparison of the protein profiles in CSF samples obtained from patients with AD with the protein profiles in CSF samples obtained from patients with FTD and control patients

2.1 Master 2D map and protein identification by MS

A total of 622 protein spots were matched between all 36 gels on which AD (AD1-AD6; Table 1), FTD (FTD1-FTD6; Table 1) and control patients (C1-C6; Table 1) were compared. A high resolution 2D protein gels (12.5 % PAGE, narrow range pI 4.5-5.5) were obtained with reproducible patterns. This allowed the comparision of CSF samples, between the two different neurological diseases and the unaffected controls. As shown in Figure 1, all differentially expressed proteins are present on the 2D master map as multiple protein species (spots). The protein spot volume levels calculated by PDQuest processing, indicate that out of 622 protein spots, 100 distinct protein spots were differentially regulated and independently related to Alzheimer's disease, Frontotemporal dementia or to healthy controls. Out of these, for 30 protein spots, a partial protein sequence was obtained. In combination with database searches the protein species were identified (Table 2). The other 70 protein spots were not identified by sequencing so far.

2.2 Changes in apolipoprotein levels

The majority of the altered CSF protein profiles (12/100) were found in the apolipoproteins group. For the apolipoprotein J, expression levels of 5 protein species were altered (Table 3). Three apo J spots (spot numbers 4401, 5302 and 8601) were down-regulated in AD patients in contrast to controls and FTD (1.8 fold increase for controls compared to AD, and up to 2 fold increase for FTD compared to AD). A qualitative difference was determined for the apo J-3405 spot, that was significantly down-regulated in FTD compared with controls (1.5 fold C/FTD $p<0.01$).

Protein species determined by MS as apolipoprotein A-IV have diagnosis-related alterations detectable on the CSF level. The most significant one is the apo AIV-4606 spot, which was down-regulated in AD (2 fold difference between FTD and AD,

$p<0.001$) and controls (2 fold difference between FTD and controls, $p<0.01$) compared to FTD.

Between the three altered apolipoprotein A-I spots, the most distinguished one is the apo AI-6102 spot, whose expression is increased 2 times in AD when compared to FTD ($p<0.03$).

Apolipoprotein E was detected in the 2D gel in two forms, at approximately 35 kDa and, as truncated isoform, at 13-15 kDa. The quantitative value of the Apo E-6502 spot isoform was significantly up-regulated in the FTD versus AD patients ($p=0.03$, 1.9 fold increase FTD/AD). The comparison of the spot volumes between AD patients and controls indicated that the Apo E-6502 spot down regulation is associated with neurological disease.

2.3 Influence of blood-brain barrier

For the expression levels of several blood derived glycoproteins mainly qualitative differences were detected in the CSF samples of patient's suffering from frontotemporal dementia. On average, FTD patients have a higher Q albumin ratio than AD or control patients. Therefore vitamin D binding protein, hemopexin, haptoglobin, gelsolin and α-1-β glycoprotein, which may originate from the blood, were selected to test whether any correlation to the Q albumin ratio was present. For the vitamin D-binding protein (3601RBH spot) quantitative and qualitative differences were established. Two isoforms of vitamin D-binding protein (spots 3601RBH and 4411, $p=0.04$) were quantified as 2.3 and 1.8 fold decrease in AD versus FTD. Qualitative analyses confirmed this data ($p=0.005$). For this protein the data provide evidence for a positive correlation between abundance and Q-albumin ratio, which explains about 35% of the variation observed.

2.4 Other disease-related protein patterns in CSF

The intensity of the hemopexin-8902RBH spot isoform was quantified to be two times lower for AD and control patients than for FTD patients ($p=0.04$, FTD>C and $p=0.06$, FTD>AD).

The expression levels between the compared groups were determined qualitatively for the alpha-1 antytrypsin-7206 spot isoform (p=0.005, AD>C), for the α-1-β glycoprotein- 4801RBH spot and 1RBH spot isoforms (p=0.03 and p=0.009, FTD/AD), for the antithrombin III-5702 spot isoform (p=0.03, FTD>C), for the haptoglobin-5004 spot isoform (p=0.01 C>FTD) and for the hemopexin-5903RBH spot isoform (p=0.04, FTD>C).

The transthyretin levels were also differentially influenced when AD and FTD patients were compared with healthy controls. For the transthyretin-7102 spot and 7108 spot isoforms, spot volume values were reproducibly and significantly decreased in AD versus FTD's (p=0.04 and 0.02, respectively). In contrast, the transthyretin-6001 spot isoform was increased in the AD patients when compared to the FTD group (1.71 fold, p=0.04).

The prostaglandin-H2 D-isomerase, abundant in the CSF and at very low concentration in the serum (16.6 mg/L in CSF versus 0.49 mg/L) is considered to be a CSF specific protein. As the prostaglandin-H2 D-isomerase-9209 spot and 8101 spot isoforms were absent in all AD gels or in all FTD gels respectively, only qualitative values were determined for these isoforms (p=0.01).

For the alpha-1-antitrypsin, antithrombin III, Apo E and Apo A-I, the identified protein isoforms were elaborated by Western blots. A Western blot with anti-Apo E M-012 (INNOGENETICS N.V., Gent, Belgium) is shown in Figure 2.

2.5 Analysis of unknown CSF samples

To verify the reproducibility and power of differential diagnosis based on differential proteomic analysis, a mock experiment was designed. In this experiment we aimed to diagnose unknown CSF sample on the basis of a 'differential 2D pattern' which we assumed to be predictable for different neurodegenerative diseases (dementia). Without knowing the diagnosis of the patients, 4 CSF samples were analyzed. The samples were processed in an identical way as is described above.

Every sample was separated twice on a 2D gel. All the gels were submitted to a spot detection wizard under the same spot detection parameters. After landmarks were added and the gels were matched to each other and to the master gel, the log10 was calculated and the data were standardized. The results of the t-test (2-tailed, equal variances), performed for every gel-patient combination and for every spot, showed a significant difference in the total spot number between the samples. These data show that the least differences were found between CSF1 and CSF 2 samples and between CSF 3 and CSF 4. Between these two groups 19 protein isoforms showed significant differences in t test analysis. When the spot intensities of these 19 protein isoforms were compared with the spot volumes of the the apolipoprotein J-3405 spot isoform, the vitamin D-binding protein-4411 and -3601RBH spot isoform, the haptoglobin-6403 spot isoform and the 5801 spot isoform (not identified) pointed out that samples CSF 3 and CSF 4 behave as previously analyzed FTD CSF samples. To confirm this assumption, all spots which scored as significant when the FTD group was compared with the 2 others groups (AD and controls), were compared with the spots in the mock experiment. At least 6 spots indicated clearly that samples CSF 3 and CSF 4 had the 'differential 2D pattern' from patients suffering Frontotemporal dementia.

Example 3: Comparison of the protein profiles in CSF samples obtained from patients with AD with the protein profiles in CSF samples obtained from patients with depression

To determine which consequence albumin and IgG depletion would have on 2D experiment, 6 CSF samples from AD patients (AD7-AD12); Table 1) were compared with CSF samples from 6 depression (D1-D6; Table 1) patients. In addition to affinity chromatography on the ABD matrix (Pharmacia), 2 ml of each CSF sample was subsequently run on a Prot A column. The total number of protein spots determined in this experiment was 1003 from which 41 were differentially regulated between the two compared groups. Due to the CSF depletion, gel to gel reproducibility of 85 % was achieved. This was a significant improvement in comparison with reproducibility of 45 % obtained in the previous experiment (example 2). The 2D gel image of the CSF samples obtained from patients with depression and on which protein isoform spots are indicated, is shown in Figure 3. An overview of the protein isoforms that

were differentially present in CSF from AD patients compared to CSF from patients with depression is shown in Table 4. The results from the first experiment (Example 2; AD-FTD-C), stating that the most prominently influenced proteins in AD CSF are apolipoproteins, were confirmed.

Example 4: Comparison of the level of different Apolipoprotein A-I isoforms in CSF samples obtained from patient with AD versus CSF samples obtained from patients with FTD, VAD or depression

Apolipoprotein A-I patterns were examined in a proteomic screening procedure in 38 patients organized in 3 experiments as described in example 1. In a first experiment, we compared the CSF proteome of 6 AD (AD1-AD6; Table 1), 6 FTD (FTD1-FTD6; Table 1) and 6 control (C1-C6; Table 1) patients. In an additional experiment 4 FTD patients were compared with 4 VAD patients (B1-B8; Table 1). In a third experiment, CSF from 6 depression (D1-D6; Table 1) and 6 AD (AD7-AD12; Table 1) patients was compared. In this third experiment, an exception was made to the standard protocol as the CSF was further depleted for human serum albumin and immunoglobulin G as described in example 3. This pre-fractionation procedure increased the reproducibility from 45% (as in experiment 1 and 2) to 85% in experiment 3.

When the Apo A-I isoforms were screened through all analyzed gels and compared between the different neurological disorders, three Apo A-I isoform spots (4310, 6102 and 7101) were differentially regulated and showed statistically significant differences between the diagnostic groups (Table 5). The most prominent isoform was the Apo AI-7101 spot which was down regulated in CSF samples obtained from Alzheimer disease patients in comparison with CSF samples obtained from patients with FTD, VAD or depression ($p<0.01$, $p<0.001$ and $p<0.08$, respectively). The isoform 6102 spot levels were upregulated in AD patients when compared with FTD and VAD patients ($p<0.01$ and $p<0.001$).

Example 5: Comparison of the level of different Apolipoprotein E isoforms in CSF samples obtained from patient with AD, FTD, VAD or depression or from healthy controls

After albumin and IgG were removed from the CSF (as described in example 3), the gel area where Apolipoprotein E was previously determined became more assessable for spot identification. In total 12 Apo E isoforms were identified (Table 6), from which 4 'full size' Apo E isoforms (isoform spot numbers 110, 114, 480 and 862) and 8 truncated forms (isoform spot number 180, 183, 263, 271, 272, 274, 462 and 681). The full size isoforms displayed significant differences in expression levels. Three full size isoforms were significantly decreased in CSF samples obtained from Alzheimer's disease patients when compared with their level in CSF samples from depression patients while one full size isoform was significatnly increased in CSF samples obtained from AD patients compared to CSF samples obtained from patients with depression. In the comparision between CSF samples from AD patients and VAD patients, Apo E isoform spots 274 and 263 were significantly down regulated in patients suffering AD disease.

TABLES

Table 1. Overview of the CSF samples used in the 2D analysis. CSF samples were clinically diagnosed as AD (AD1-AD12), FTD (FTD1-FTD6, B3, B4, B7 and B8), healthy controls (C1-C6), VAD (B1, B2, B5 and B6) and depression (D1- D6).

Patient code	Diagnosis	Sex	Age	MMSE	ApoE	Tau (pg/ml)	Ab42 (pg/ml)	Albumin ratio
AD1	AD	f	78	22	4+4	545	299	3.63
AD2	AD	m	72	18	4+3	1720	380	3.84
AD3	AD	f	78	26	4+3	764	448	3,6
AD4	AD	m	74	21	4+4	728	460	5.32
AD5	AD	f	76	26	4+3	508	398	4.21
AD6	AD	m	74	25	4+3	1220	418	5.5
AD7	AD	f	79	19	4+4	710	288	4.8
AD8	AD	f	88	18	3+3	592	440	6.6
AD9	AD	f	75	21	4+3	516	499	4.6
AD10	AD	f	89	17	4+3	741	390	6.9
AD11	AD	m	88	18	4+3	636	324	6.4
AD12	AD	m	76	18	4+4	599	395	3.3
C1	Control	m	64	30	3+2	251	1070	2.93
C2	Control	m	67	25	4+3	342	1084	5.77
C3	Control	m	53	30	3+3	204	1079	3.97
C4	Control	f	62	30	3+3	266	1125	3.42
C5	Control	m	60	30	3+3	298	1108	4.31
C6	Control	f	81	27	3+3	224	636	5.83
FTD1	FTD	f	69	18	3+3	444	828	4.50
FTD2	FTD	f	66	18	3+3	401	642	6.90
FTD3	FTD	f	76	22	3+3	363	833	5.90
FTD4	FTD	m	69	18	4+4	471	218	5.80
FTD5	FTD	f	62	15	NA	214	NA	7.70
FTD6	FTD	m	71	16	4+3	348	823	7.80
B1	VAD	m	71	8	NA	573	303	6.8
B2	VAD	f	79	NA	NA	NA	NA	NA
B3	FTD	f	71	25	3+3	388	148	16
B4	FTD	f	63	19	3+3	737	257	5.9
B5	VAD	m	73	22	NA	318	213	6
B6	VAD	m	76	19	NA	244	718	6.3
B7	FTD	m	68	25	3+3	778	510	5.5
B8	FTD	m	47	25	4+3	430	644	10.4
D1	Depression	m	69	28	3+3	256	730	5.4
D2	Depression	f	81	27	3+3	330	558	4.8
D3	Depression	f	76	29	NA	514	NA	10.6
D4	Depression	m	79	27	NA	208	NA	7
D5	Depression	f	44	30	3+3	342	1089	6.1
D6	Depression	f	75	25	3+3	357	900	3.93

NA: not available.

Table 2. Identification of the protein spots that were altered between the studied groups.

Spot exp1	Spot exp2	Peptide	aa	Identification in database	ID number database
2713	674	TDTSHHDQDHPTFNK	35-49	Alpha-1-antitrypsin	P01009
		LVDKFLEDVK	150-159		
		FLEDVKK	154-160		
		KQINDYVEK	179-187		
		QINDYVEK	180-187		
		DTEEEDFHVDQATTVK (M1A allele)	226-241		
		DTEEEDFHVDQVTTVK (M1V allele)	226-241		
		LQHLENELTHDIITK	284-298		
		FLENEDR	299-305		
		FLENEDRR	299-306		
		SASLHLPK	307-314		
		LSITGTYDLK	315-324		
		SVLGQLGITK	325-334		
		VFSNGADLSGVTEEAPLK	335-352		
		AVLTIDEK	360-367		
4704	353	TDTSHHDQDHPTFNK	35-49	Alpha-1-antitrypsin	P01009
		FLEDVKK	154-160		
		KQINDYVEK	179-187		
		QINDYVEK	180-187		
		DTEEEDFHVDQATTVK (M1A allele)	226-241		
		DTEEEDFHVDQVTTVK (M1V allele)	226-241		
		LSSWVLLMK + 1 Oxidation (M)	259-267		
		FLENEDR	299-305		
		FLENEDRR	299-306		
		SASLHLPK	307-314		

		LSITGTYDLK	315-324		
		SVLGQLGITK	325-334		
		VFSNGADLSGVTEEAPLK	335-352		
		AVLTIDEK	360-367		
		LGMFNIQHCK (Cys-CAM)	248-257		
4705	355	QINDYVEK	180-187	Alpha-1-antitrypsin	P01009
7206		LSITGTYDLK	315-324	Alpha-1-antitrypsin	P01009
		SVLGQLGITK	325-334		
		VFSNGADLSGVTEEAPLK	335-352		
4801RBH		LLELTGPK	86-93	Alpha-1B-glycoprotein	P04217
4803	375	ATWSGAVLAGR	386-396	Alpha-1B-glycoprotein	P04217
		FALVREDR	313-320		
1RBH		CLAPLEGAR (cys-CAM + ox)	304-312	Alpha-1B-glycoprotein	P04217
		FALVREDR	313-320		
901RBH		LETPDFQLFK	32-41	Alpha-1B-glycoprotein	P04217
		ATWSGAVLAGR	386-396		
		LLELTGPK	86-93		
5702		EVPLNTIIFMGR + 1 Oxidation (M)	446-457	Antithrombin-III	P01008
6102	149	DYVSQFEGSALGK	52-64	Apolipoprotein A-I	P02647
		VQPYLDDFQK	121-130		
		$[916.56]^{+}$ALKED$[360.25]^{+}$ (aa 208 : N → D)	201-212		
		ATEHLSTLSEK	220-230		
		AKPALEDLR	231-239		
		LSPLGEEMR + 1 Oxidation (M)	165-173		
		THLAPYSDELR	185-195		
		LLDNWDSVTSTFSK	70-83		
		DSGRDYVSQFEGSALGK	48-64		
		LEALKENGGAR	202-212		
		QGLLPVLESFK	240-250		

		VEPLRAELQEGAR	143-155		
6303	146	DYVSQFEGSALGK	52-64	Apolipoprotein A-I	P02647
		VQPYLDDFQK	121-130		
		$[916.56]^{+}$ALKED$[360.27]^{+}$ (aa208 : N → D)	201-212		
		ATEHLSTLSEK	220-230		
		LSPLGEEMRDR + 1 Oxidation (M)	165-175		
		LSPLGEEMR + 1 Oxidation (M)	165-173		
		THLAPYSDELR	185-195		
7101	285	DSGRDYVSQFEGSALGK	48-64	Apolipoprotein A-I	P02647
		DYVSQFEGSALGK	52-64		
		$[1315.83]^{+}$DNDDSVTSTFSK (aa 74: W→D)	70-83		
		QEMSKDLEEVK + 1 Oxidation (M)	108-118		
		VQPYLDDFQK	121-130		
		VQPYLDDFQKK	121-131		
		LSPLGEEMR + 1 Oxidation (M)	165-173		
		LSPLGEEMRDR + 1 Oxidation (M)	165-175		
		THLAPYSDELR	185-195		
		LEALKED$[360.25]^{+}$ (aa 208 : N → D)	202-212		
		LEALKENGGAR	202-212		
		ATEHLSTLSEK	220-230		
		AKPALEDLR	231-239		
		DLATVYVDVLK	237-247		
		QGLLPVLESFK	240-250		
		QKLHELQEK (E → pyroglutamic acid)	156-164		
		VEPLRAELQEGAR	143-155		
		LLDNWDSVTSTFSK	70-83		
		DEPPQSPWDR + 1 Oxidation (W)	25-34		
		DLATVYVDVLK	37-47		
		VSFLSALEEYTK	251-262		

		KWQEEMELYR + 1 Oxydation (M)	131-140		
4310	144	THLAPYSDELR	185-195	Apolipoprotein A-I	P02647
	143	THLAPYSDELR	185-195	Apolipoprotein A-I	P02647
4606+4605		LGEVNTYAGDLQK	66-78	Apolipoprotein A-IV	P06727
		LLPHANEVSQK	113-123		
		QLTPYAQR	156-163		
		IDQNVEELKGR	190-200		
		LTPYADEFK	201-209		
		ISASAEELR	256-264		
		LAPLAEDVR	267-275		
		ALVQQMEQLR + 1 Oxidation (M)	317-326		
5402	81	LEPYADQLR	135-143	Apolipoprotein A-IV	P06727
		IDQNVEELKGR	190-200		
		LTPYADEFK	201-209		
		IDQTVEELR	212-220		
		ISASAEELR	257-264		
		LAPLAEDVR	267-275		
		ALVQQMEQLR + 1 Oxidation (M)	317-326		
		RVEPYGENFNK	306-317		
		SLAPYAQDTQEK	222-233		
		LGEVNTYAGDLQK	66-78		
6502		KVEQAVETEPEPELR	19-33	Apolipoprotein E	P02649
		AYKSELEEQLTPVAEETR	91-109		
		LSKELQAAQAR	111-121		
		AATVGSLAGQPLQER	210-224		
		AKLEEQAQQIR	259-269		
		LQAEAFQAR	270-278		
5502	110	LQAEAFQAR	270-278	Apolipoprotein E	P02649
		AKLEEQAQQIR	259-269		

		LLRDADDLQK	166-175		
		KVEQAVETEPEPELR	19-33		
	114	LQAEAFQAR	270-278	Apolipoprotein E	P02649
		AKLEEQAQQIR	259-269		
		LGPLVEQGR	199-207		
		VQAAVGTSAAPVPSDNH	301-317		
	272	LGPLVEQGR	199-207	Apolipoprotein E	P02649
	862	LGPLVEQGR	199-207	Apolipoprotein E	P02649
	681	KVEQAVETEPEPELR	19-33	Apolipoprotein E	P02649
	480	LQAEAFQAR	270-278	Apolipoprotein E	P02649
		LGPLVEQGR	199-207		
		AKLEEQAQQIR	259-269		
		ALMDETMKELK + 2 Oxidations (M)	80-90		
3405		ELDESLQVAER	326-336	Apolipoprotein J	P10909
3505		ELDESLQVAER	326-336	Apolipoprotein J	P10909
4401	323	ELDESLQVAER	326-336	Apolipoprotein J	P10909
		KYNELLK	340-346		
		FMETVAEK + 1 Oxidation (M)	430-437		
5302	108	ELDESLQVAER	326-336	Apolipoprotein J	P10909
		EILSVDCSTNNPSQAK + 1 (cys-CAM)	307-322		
8601		TLLSNLEEAK	69-78	Apolipoprotein J	P10909
		IDSLLENDR	159-167		
		ASSIIDELFQDR	183-194		
5202		AGALNSNDAFVLK	585-597	Gelsolin	P06396
		YIETDPANR	730-738		
6404		AGALNSNDAFVLK	585-597	Gelsolin	P06396
		TGAQELLR	616-623		
5004	411	TEGDGVYTLNDKK	60-72	Haptoglobin-1/2	P00737
			119-131		P00738

		TEGDGVYTLNDKKQWINK + 1 ox (W)	60-77		P00737
			119-136		P00738
5903RBH		NFPSPVDAAFR	92-102	Hemopexin	P02790
		GGYTLVSGYPK	333-343		
8902RBH		NFPSPVDAAFR	92-102	Hemopexin	P02790
		QGHNSVFLIK	103-112		
		DYFMPCPGR + 1 (cys-CAM + ox) + 1 ox (M)	226-234		
		GGYTLVSGYPK	333-343		
4701RBH		SAVQGPPER	169-177	Ig alpha-1 chain C region (heavy)	P01876
		QEPSQGTTTFAVTSILR	283-299		
4804		TPLTATLSK	213-221	Ig alpha-1 chain C region (heavy)	P01876
4702		TVGSDTFYSFK	65-75	Kininogen	P01042
		QVVAGLNFR	188-196		
		YFIDFVAR	317-324		
8101		APEAQVSVQPNFQQDK	23-38	Prostaglandin-H2 D-isomerase	P41222
		TMLLQPAGSLGSYSYR + 1 Oxidation (M)	93-108		
		AQGFTEDTIVFLPQTDK	169-185		
9209		$[1617.85]^{+}$EAQVSVQPNF$[518.26]^{+}$	23-38	Prostaglandin-H2 D-isomerase	P41222
		TMLLQPAGSLGSYSYR + 1 Oxidation (M)	93-108		
6001		AADDT**D**EPFASGK (aa 61 : W → D)	56-68	Transthyretin	P02766
7102		[603.41]PLMVK	21-35	Transthyretin	P02766
7108	274	GPTGTGESKCPLMVK ($Cys(O_3H)$)	21-35	Transthyretin	P02766
		GPTGTGESKCPLMVK ($Cys(O_3H)$/ M : oxidation to sulphone)	21-35		
		AADDTWEPFASGK (W + 2*16 Da)	56-68		
		AADDT**D**EPFASGK (aa 61 : W → D)	56-68		
		AADDTWEPFASGK	56-68		
		KAADDTWEPFASGK	55-68		
		TSESGELHGLTTEEEFVEGIYK	69-90		

3601RBH		HLSLLTTLSNR	208-218	Vitamin D-binding protein	P02774
		YTFELSR	346-352		
		THLPEVFLSK	354-363		
		VLEPTLK	364-370		
		ELSSFIDK	395-402		
4411		VCSQYAAYGEK (cys-CAM + ox)	219-229	Vitamin D-binding protein	P02774
		VMDKYTFELSR + 1 Oxidation (M)	342-352		
		YTFELSR	346-352		
		THLPEVFLSK	354-363		
		VLEPTLK	364-370		
		[1433.61]$^{+}$CCDVEDSTTCFNAK (1 cys-CAM + ox, 2 Dha)	371-388		
		ELSSFIDK	395-402		
		AKLPDATPK	428-436		
2402	88	AGEVQEPELR	239-248	Zinc-alpha-2-glycoprotein	P25311
		QDPPSVVVTSHQAPGEK	201-217		

Table 3. Proteins identified on a 2D-gel that were significantly altered in CSF obtained from patients with AD, FTD and/or controls (C). Each protein spot has an apparent molecular mass value and an approximate pI value respective to the gel region in which it was identified.

NPI	Spot number	Protein	Mw (kDa)	pI	ID number database	p-value QN	p-value QL	Increase	
1	7206	α-1-antitrypsin	15.1	5.30	P01009		0.005	AD/C	2.3 fold
2	4801RBH	α-1β-glycoprotein	72.5	5.32	P04217		0.03	FTD/AD	1.4 fold
3	1RBH	α-1β-glycoprotein	42.1	5.13	P04217		0.009	FTD/AD	2.1 fold
4	5702	Antithrombin-III	62	5.12	P01008		0.03	FTD/C	1.5 fold
5	6102	Apolipoprotein A-I	24.5	5.22	P02647	0.03		AD/FTD	2 fold
						0.005		C/FTD	2.7 fold
6	6303	Apolipoprotein A-I	24.0	5.14	P02647		0.03	AD/FTD	1.1 fold
							0.03	FTD/C	1 fold
7	7101	Apolipoprotein A-I	24.2	5.27	P02647	0.03		FTD/AD	1.5 fold
8	4606	Apolipoprotein A-IV	43.4	5.08	P06727	0.001		FTD/AD	2 fold
						0.01		FTD/C	2 fold
9	5402	Apolipoprotein A-IV	43.4	5.14	P06727	0.04		FTD/AD	1.5 fold
						0.03		FTD/C	1.5 fold
10	4605	Apolipoprotein A-IV	42.7	5.08	P06727	0.04		FTD/C	1.6 fold
11	6502	Apolipoprotein E	35.3	5.22	P02649	0.03		FTD/AD	1.9 fold
12	3405	Apolipoprotein J	29.1	4.98	P10909		0.01	C/FTD	1.5 fold
13	3505	Apolipoprotein J	35.3	5.00	P10909		0.03	AD/FTD	1.2 fold
14	4401	Apolipoprotein J	36.6	5.07	P10909	0.009		FTD/AD	2 fold
15	5302	Apolipoprotein J	36.3	5.18	P10909	0.01		FTD/AD	1.8 fold
16	8601	Apolipoprotein J	35.2	5.35	P10909	0.02		C/AD	1.8 fold
							0.04	FTD/AD	1.2 fold
17	5202	Gelsolin	29.9	5.16	P06396	0.04		C/FTD	1.9 fold

Table 3. Continued

NPI	Spot number	Protein	MW (kDa)	pI	ID number database	p-value QN	QL	Increase	
18	5004	Haptoglobin-1/2	12.5	5.19	P00737		0.01	C/FTD	1.2 fold
					P00738				
19	5903RBH	Hemopexin	77.1	5.37	P02790		0.04	FTD/C	1.4 fold
20	8902RBH	Hemopexin	75.9	5.46	P02790	0.04		FTD/C	2.2 fold
						0.06		FTD/AD	2.5 fold
21	4701RBH	Ig α-1 chain C region (heavy)	66.7	5.31	P01876		0.01	FTD/AD	1.3 fold
22	4804	Ig α-1 chain C region (heavy)	66.6	5.09	P01876		0.0001		
							0.01	FTD/C	1.9 fold
23	4702	Kininogen	65.6	5.06	P01042	0.03		FTD/C	1.7 fold
24	8101	Prostaglandin-H2 D-isomerase	24.5	5.34	P41222		0.01	C/AD	1.7 fold
25	9209	Prostaglandin-H2 D-isomerase	27.3	5.44	P41222		0.01	C/FTD	1.1 fold
26	6001	Transthyretin	12.7	5.22	P02766	0.04		AD/FTD	1.7 fold
27	7102	Transthyretin	20.5	5.28	P02766	0.04		FTD/AD	1.6 fold
28	7108	Transthyretin	13.8	5.24	P02766	0.02		FTD/AD	1.4 fold
						0.05		FTD/C	1.6 fold
29	3601RBH	Vitamin D-binding protein	57.0	5.29	P02774	0.04		FTD/AD	2.3 fold
							0.005		
30	4411	Vitamin D-binding protein	32.5	5.06	P02774	0.04		FTD/AD	1.8 fold
							0.005		

QN: quantitative detection; QL: qualitative detection.

Table 4. Proteins identified on a 2D-gel that significantly altered in CSF from patients suffering AD compared to patients suffering depression (D). Each protein spot has an apparent molecular mass value and an approximate pI value respective to the gel region in which it was identified.

NPI	Spot no.	Protein	MW (kDa)	pI	ID number database	p-value		Comparison		
						QN	QL	D/AD	AD/D	
31	**32**	α-1-β-glycoprotein	79,2	5,18	P04217	0,0406	ns	7,75	0,13	AD < D
23	**45**	Kininogen	65,6	5,06	P01042	0,0377	ns	5,11	0,20	AD < D
32	**61**	NI	57,4	5,08	-	0,0341	ns	6,41	0,16	AD < D
33	**88**	Zn-α-2-glycoprotein	42,1	4,88	P25311	0,0160	ns	7,20	0,14	AD < D
34	**110**	Apolipoprotein E	35,2	5,11	P02649	0,0025	ns	62,26	0,02	AD < D
35	**114**	Apolipoprotein E	34,3	5,32	P02649	0,0385	ns	23,95	0,04	AD < D
36	**120**	NI	32,4	4,9	-	/	0,0373	/	/	AD < D
37	**143**	Apolipoprotein A-I	24	5,1	P02647	0,0408	ns	6,75	0,15	AD < D
6	**146**	Apolipoprotein A-I	24	5,14	P02647	0,0244	ns	10,50	0,10	AD < D
38	**173**	NI	17,8	5,13	-	/	0,0137	/	/	AD < D
39	**178**	NI	16,7	5,01	-	0,0032	ns	0,25	4,02	AD > D
40	**184**	NI	15,6	5,24	-	0,0329	ns	7,36	0,14	AD < D
41	**272**	Apolipoprotein E	15,3	5,07	P02649	0,0218	ns	2,90	0,34	AD < D
42	**348**	α-1-antitrypsin	58,5	4,92	P01009	0,0094	ns	9,67	0,10	AD < D
43	**353**	α-1-antitrypsin	57,3	5,05	P01009	0,0232	ns	7,11	0,14	AD < D
44	**355**	α-1-antitrypsin	57,7	5	P01009	0,0294	ns	6,74	0,15	AD < D
45	**366**	NI	63,1	5,12	-	/	0,0272	1,07	0,94	AD < D
46	**370**	NI	53,1	5,23	-	/	0,0361	1,14	0,88	AD < D
47	**377**	NI	82,1	5,03	-	0,0273	ns	5,72	0,17	AD < D
48	**388**	α-1-β-glycoprotein	79,2	5,18	P04217	0,0120	ns	10,96	0,09	AD < D
49	**409**	NI	15,1	5,17	-	/	0,0001	/	/	AD > D
50	**465**	NI	13,8	5,37	-	0,0481	ns	0,28	3,57	AD > D

Table 4. Continued

NPI	Spot no.	Protein	MW (kDa)	pI	ID number	p-value	Difference	Comparison		
						QN	QL	D/AD	AD/D	
51	**470**	NI	50,7	5,13	-	0,0212	ns	3,62	0,28	AD < D
52	**480**	Apolipoprotein E	33,8	5,3	P02649	0,0307	ns	24,21	0,04	AD < D
53	**482**	NI	56,5	5,15	-	/	0,0373	/	/	AD < D
54	**486**	NI	15,6	5,32	-	/	0,0391	1,14	0,88	AD < D
55	**513**	NI	13,7	5,4	-	/	0,0272	2,40	0,42	AD < D
56	**599**	NI	82,7	4,99	-	0,0394	ns	11,08	0,09	AD < D
57	**639**	NI	57,7	4,52	-	/	0,0137	/	/	AD > D
58	**646**	NI	55,5	5,12	-	0,0013	ns	11,90	0,08	AD < D
59	**674**	α-1-antitrypsin	58,5	4,92	P01009	0,0228	ns	9,33	0,11	AD < D
60	**681**	Apolipoprotein E	15,6	5,38	P02649	0,0182	ns	4,63	0,22	AD < D
61	**699**	NI	31,4	5,09	-	/	0,0373	/	/	AD < D
62	**729**	NI	17	5,4	-	0,0259	ns	0,23	4,39	AD > D
12	**748**	Apolipoprotein J	30,3	5,01	P10909	0,0246	ns	2,62	0,38	AD < D
63	**798**	NI	35,4	5,14	-	0,0236	ns	4,48	0,22	AD < D
64	**813**	NI	30,2	5,38	-	0,0321	ns	0,36	2,81	AD > D
65	**839**	NI	13,8	4,64	-	0,0332	ns	0,50	1,98	AD > D
66	**862**	Apolipoprotein E	30	5,17	P02649	/	0,0373	/	/	AD > D
67	**968**	NI	33,1	5,37	-	/	0,0361	0,56	1,79	AD > D
68	**1004**	NI	15,7	5,37	-	/	0,0373	/	/	AD < D

NI: not identified; ns: no statistically significant difference was found; /: not applicable.
QN: quantitative detection; QL: qualitative detection.

Table 5. Apolipoprotein A-I isoforms that were altered in CSF samples isolated from AD patients compared to CSF samples isolated from patients suffering FTD, VAD or depression (D).

NPI	Apo A-I isoforms	PI	MW (kDa)	12 AD vs. 10 FTD	p-value	12 AD vs. 4 VAD	p-value	6 AD vs. 6 D	p-value
69	4310*	5.08	24.3	AD<FTD	0.02	AD>VAD	0.08	AD<D	0.04
6	6303	5.14	24	AD>FTD	0.34	AD>VAD	/	AD<D	0.02
70	5102	5.16	24.2	AD<FTD	0.07	AD<VAD	0.11	AD<D	0.07
5	6102	5.22	24.5	AD>FTD	0.04	AD>VAD	0.004	AD<D	0.05
7	7101	5.27	24.2	AD<FTD	0.01	AD<VAD	0.001	AD<D	0.08
71	8105	5.35	20.2	AD<FTD	0.01	AD~VAD	/	/	/
37	143*	5.10	24	AD<FTD	0.02	AD>VAD	0.08	AD<D	0.04

*Spot numbers 4310 and 143 are not always resolved as a single spot on 2D gel.

Table 6. Comparision of the level of Apolipoprotein E isoforms in CSF samples isolated from patients suffering AD, FTD, VAD or depression (D) or from healthy controls.

NPI	Apo E isoform (Exp1)	Apo E isoform (Exp2)	MW (kDa)	pI	ID No.	Ratio AD/FTD/C	Ratio AD/VAD	Ratio D/AD	Aa start-end	Peptide sequence
34	5502	110	35.2	5.11	P02649	/	AD>VAD	D>AD*	270-278	LQAEAFQAR
									259-269	AKLEEQAQQIR
									166-175	LLRDADDLQK
									19-33	KVEQAVETEPEPELR
35	/	114	34.3	5.32	P02649	/	/	D>AD*	270-278	LQAEAFQAR
									259-269	AKLEEQAQQIR
									199-207	LGPLVEQGR
									301-317	VQAAVGTSAAPVPSDNH
72	4205	180	15.8	5.07	P02649	/	/	D>AD	199-207	LGPLVEQGR
									270-278	LQAEAFQAR
									259-269	AKLEEQAQQIR
73	4103	183	15.8	5.11	P02649	FTD>AD>C	AD>VAD	D>AD	270-278	LQAEAFQAR
74	2206	263	15.8	4.91	P02649	C>FTD>AD	AD<VAD*	AD>D	199-207	LGPLVEQGR
									270-278	LQAEAFQAR
									259-269	AKLEEQAQQIR
									210-224	AATVGSLAGQPLQER
									138-152	GEVQAMLGQSTEELR
									94-108	SELEEQLTPVAEETR
									301-317	VQAAVGTSAAPVPSDNH
75	/	271	15.1	5.09	P02649	/	/	D>AD	138-152	GEVQAMLGQSTEELR
									19-33	KVEQAVETEPEPELR
									270-278	LQAEAFQAR

Table 6. Continued

41	/	272	15.3	5.07	P02649	/	/	D>AD*	19-33	KVEQAVETEPEPELR
									270-278	LQAEAFQAR
76	7108 (m)	274	13.8	5.24	P02649	C<AD<FTD	AD<VAD*	D>AD	210-224	AATVGSLAGQPLQER
77	2005 (m)	462	12.4	4.96	P02649	AD>FTD>C	AD>VAD	AD>D	259-269	AKLEEQAQQIR
52	/	480	33.8	5.30	P02649	/	/	D>AD*	270-278	LQAEAFQAR
									199-207	LGPLVEQGR
									259-269	AKLEEQAQQIR
									80-90	ALMDETMKELK
60	/	681	15.6	5.38	P02649	/	/	D>AD*	19-33	KVEQAVETEPEPELR
66	/	862	30.0	5.17	P02649	/	/	AD>D*	199-207	LGPLVEQGR
11	6502	/	35.3	5.22	P02649	FTD>C>AD	/	/	19-33	KVEQAVETEPEPELR
									91-109	AYKSELEEQLTPVAEETR
									111-121	LSKELQAAQAR
									210-224	AATVGSLAGQPLQER
									259-269	AKLEEQAQQIR
									270-278	LQAEAFQAR

* CSF-Levels of the protein isoforms were statistical significant ($p<0.05$) different in AD patients compared to patients with VAD or depression respectively.

Table 7. Overview of antibodies that are available for use in the immunoassay of the invention.

Antibody	**Manufacturer**
Mab anti-human Apolipoprotein E A1.4	Santa Cruz, Biotecnology, Inc, Santa Cruz, CA, US
Polyclonal anti-human Apolipoprotein E E-19	Santa Cruz, Biotecnology, Inc, Santa Cruz, CA, US
Mab anti-human Apolipoprotein E 3D12	MEDICAL AND BIOLOGICAL LABORATORIES CO. Lnaka-ku Nagoya, Japan
Polyclonal anti-human Apolipoprotein E A0077	DAKO A/S, Glostrup, Denmark
Mab anti-human Apolipoprotein E D6E10	SIGNET Laboratories, Inc. Dedham, MA, US
Mab anti-human Apolipoprotein E 13F4B5	INNOGENETICS N. V., Gent, Belgium
Mab anti-human Apolipoprotein E4 1F9	MEDICAL AND BIOLOGICAL LABORATORIES CO. Lnaka-ku Nagoya, Japan
Polyclonal anti-human Apolipoprotein AI, ab 7613	Abcam Ltd, Cambridge, UK
Polyclonal anti-human Folate-binding protein, ab 2107	Abcam Ltd, Cambridge, UK
Polyclonal Anti-human Apolipoprotein J, ab 7621	Abcam Ltd, Cambridge, UK
Polyclonal anti-MTI-MMP Hemopexin Domain (MMP-14)	CHEMICON INTERNATIONAL Temecula, CA, US
Polyclonal anti human Alpha -1-antitrypsin A0012	DAKO A/S, Glostrup, Denmark
Polyclonal anti-human Gelsolin	Biogenesis Ltd, England, UK
Polyclonal anti-human Antithrombin III	DAKO A/S, Glostrup, Denmark

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CLAIMS

1. A method for the screening, diagnosis and/or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, said method comprising the following steps:

   (a) detecting, in said mammal, the level of at least one of the following proteins: α-1-antitrypsin, α-1-β glycoprotein, antithrombin III, Apo A-I, Apo A-IV, Apo E, Apo J, gelsolin, haptoglobin, hemopexin, Ig α-1 chain C region (heavy), kininogen, prostaglandin-H2 D-isomerase, transthyretin, vitamin D-binding protein, Zn-α-2-glycoprotein, or of an isoform thereof; and

   (b) comparing the level of said at least one protein or protein isoform detected in step (a) with the level of said at least one protein or protein isoform in a control mammal; and

   (c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, an altered level of said at least one protein or protein isoform being an indication of the mammal suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

2. A method for the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins: α-1-antitrypsin, α-1-β glycoprotein, antithrombin III, Apo A-I, Apo A-IV, Apo E, Apo J, gelsolin, haptoglobin, hemopexin, Ig α-1 chain C region (heavy), kininogen, prostaglandin-H2 D-isomerase, transthyretin, vitamin D-binding protein, Zn-α-2-glycoprotein, or of an isoform thereof; and

(b) comparing the level of said at least one protein or protein isoform detected in step (a) with the level of said at least one protein or protein isoform in a mammal suffering from another neurological disease.

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

3. The method according to any of claims 1 to 2 further characterised that the level of at least one of the following neurological disease-associated protein isoforms is detected (Table 3; Table 4; Table 5; Table 6):

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;
- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;

- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68.

4. The method of claim 1 for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 11, NPI 16, NPI 24, NPI 74;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform being an indication of the mammal suffering from Alzheimer's disease.

5. The method of claim 1 for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least on of the following protein isoforms (Table 3; Table 6): NPI 1, NPI 73, NPI 76, NPI 77;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, an increased level of said at least one protein isoform being an indication of the mammal suffering from Alzheimer's disease.

6. The method of claim 1 for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 5, NPI 12, NPI 17, NPI 18, NPI 25, NPI 74;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Frontotemporal dementia, a decreased level of said at least one protein isoform being an indication of the mammal suffering from Frontotemporal dementia.

7. The method of claim 1 for the screening, diagnosis or prognosis in a mammal of Frontotemporal dementia, for identifying a mammal at risk of developing Frontotemporal dementia, or for monitoring the effect of therapy administered to a mammal having Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following protein isoforms (Table 3; Table 6): NPI 4, NPI 6, NPI 8, NPI 9, NPI 10, NPI 11, NPI 19, NPI 20, NPI 22, NPI 23, NPI 28, NPI 73, NPI 76, NPI 77;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a control mammal; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Frontotemporal dementia, an increased level of said at least one protein isoform being an indication of the mammal suffering from Frontotemporal dementia.

8. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 3; Table 6): NPI 5, NPI 6, NPI 13, NPI 26, NPI 77;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the is suffering from Alzheimer's disease, an increased level of said at least one isoform, compared to its level in a mammal suffering from Frontotemporal dementia, being an indication of the mammal suffering from Alzheimer's disease.

9. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus Frontotemporal dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 3; Table 5; Table 6): NPI 2, NPI 3, NPI 7, NPI 8, NPI 9, NPI 11, NPI 14, NPI 15, NPI 16, NPI 20, NPI 21, NPI 27, NPI 28, NPI 29, NPI 30, NPI 37, NPI 69, NPI 70, NPI 71, NPI 73, NPI 74, NPI 76;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering Frontotemporal dementia; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in a mammal suffering from Frontotemporal dementia, being an indication of the mammal suffering from Alzheimer's disease.

10. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 5, Table 6): NPI 5, NPI 6, NPI 7, NPI 12, NPI 23, NPI 31, NPI 32, NPI 33, NPI 34, NPI 35, NPI 36, NPI 37, NPI 38, NPI 40, NPI 41, NPI 42, NPI 43, NPI 44, NPI 45, NPI 46, NPI 47, NPI 48, NPI 51, NPI 52, NPI 53, NPI 54, NPI 55, NPI 56, NPI 58, NPI 59, NPI 60, NPI 61, NPI 63, NPI 68, NPI 69, NPI 70, NPI 72, NPI 73, NPI 75, NPI 76;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering depression; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in a mammal suffering from depression, being an indication of the mammal suffering from Alzheimer's disease.

11. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus depression, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 4; Table 6): NPI 39, NPI 49, NPI 50, NPI 57, NPI 62, NPI 64, NPI 65, NPI 66, NPI 67, NPI 74, NPI 77;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering depression; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, an increased level of said at least one protein isoform, compared to its level in a mammal suffering from depression, being an indication of the mammal suffering from Alzheimer's disease.

12. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 5, Table 6): NPI 7, NPI 70, NPI 74, NPI 76;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in a mammal suffering from Vascular dementia, being an indication of the mammal suffering from Alzheimer's disease.

13. The method of claim 2 for the differential diagnosis in a mammal of Alzheimer's disease versus Vascular dementia, said method comprising the following steps:

(a) detecting, in said mammal, the level of at least one of the following proteins isoforms (Table 5, Table 6): NPI 5, NPI 6, NPI 34, NPI 37, NPI 69, NPI 73, NPI 77;

(b) comparing the level of said at least one protein isoform detected in step (a) with the level of said at least one protein isoform in a mammal suffering Vascular dementia; and

(c) concluding from the comparison in step (b) whether the mammal is suffering from Alzheimer's disease, a decreased level of said at least one protein isoform, compared to its level in a mammal suffering from Vascular dementia, being an indication of the mammal suffering from Alzheimer's disease.

14. The method according to any of claims 1 to 13, further characterised that said method is carried out *in vitro*, on a sample obtained from said mammal.

15. The method according to claim 14 further characterised that said sample is taken from the cerebrospinal fluid or serum of said mammal.

16. A preparation comprising at least one of the following isolated protein isoforms associated with Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression:

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;
- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;

- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68.

17. A method for the detection of the level of a protein isoform according to claim 16.

18. The method according to any of claims 1 to 15 or the method according to claim 17, characterised in that the level of protein or protein isoform is detected by isoelectric focussing followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

19. The method according to any of claims 1 to 15 or the method according to claim 17, characterised in that the level of protein or protein isoform is detected by an immunoassay.

20. The method according to claim 19, further characterised that the detection of the level of protein or protein isoform comprises:

(a) contacting the protein or protein isoform with an antibody that specifically recognizes the protein or protein isoform under conditions being suitable for producing an antigen-antibody comples; and

(b) detecting the immunological binding that has occurred between the antibody and the protein or protein isoform.

21. An antibody capable of specifically recognizing one of the following protein isoforms associated with Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression:

- α-1-antitrypsin: NPI 1, NPI 42, NPI 43, NPI 44, NPI 59;
- α-1-β glycoprotein: NPI 2, NPI 3, NPI 31, NPI 48;
- Antithrombin-III: NPI 4;
- Apo A-I: NPI 5, NPI 6, NPI 7, NPI 37, NPI 69, NPI 70, NPI 71;
- Apo A-IV: NPI 8, NPI 9, NPI 10;

- Apo E: NPI 11, NPI 34, NPI 35, NPI 41, NPI 52, NPI 60, NPI 66, NPI 72, NPI 73, NPI 74, NPI 75, NPI 76, NPI 77;
- Apo J: NPI 12, NPI 13, NPI 14, NPI 15, NPI 16;
- Gelsolin: NPI 17;
- Haptoglobin: NPI 18;
- Hemopexin: NPI 19, NPI 20;
- Ig α-1 chain C region (heavy): NPI 21, NPI 22;
- Kininogen: NPI 23;
- Prostaglandin-H2 D-isomerase: NPI 24, NPI 25;
- Transthyretin: NPI 26, NPI 27, NPI 28;
- Vitamin D-binding protein: NPI 29, NPI 30;
- Zn-α-2-glycoprotein: NPI 33;
- NPI 32, NPI 36, NPI 38, NPI 39, NPI 40, NPI 45, NPI 46, NPI 47, NPI 49, NPI 50, NPI 51, NPI 53, NPI 54, NPI 55, NPI 56, NPI 57, NPI 58, NPI 61, NPI 62, NPI 63, NPI 64, NPI 65, NPI 67, NPI 68.

22. A kit comprising an antibody according to claim 21.

23. The antibody according to claim 21 or the kit according to claim 22 for use in the screening, diagnosis or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

24. The antibody according to claim 21 or the kit according to claim 22 for use in the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

25. Use of the antibody according to claim 21 for the preparation of a kit for the screening, diagnosis or prognosis in a mammal of Alzheimer's disease,

Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, for identifying a mammal at risk of developing Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

26. Use of the antibody according to claim 21 for the preparation of a kit for the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or of depression.

27. A method of screening for agents that interact with and/or modulate the expression or activity of a protein or protein isoform according to claim 16, said method comprising:

(a) contacting said protein or protein isoform or a portion of said protein or protein isoform with said agent; and

(b) determining whether or not said agent interacts with and/or modulates the expression of said protein or protein isoform or said portion of the protein or protein isoform.

ABSTRACT

A method is provided for the screening, diagnosis and/or prognosis of neurological diseases. More specifically, new biomarkers are provided for the screening, diagnosis and/or prognosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression. The method further provides for the differential diagnosis in a mammal of Alzheimer's disease, Frontotemporal dementia, dementia with Lewy bodies, Vascular dementia and/or depression.

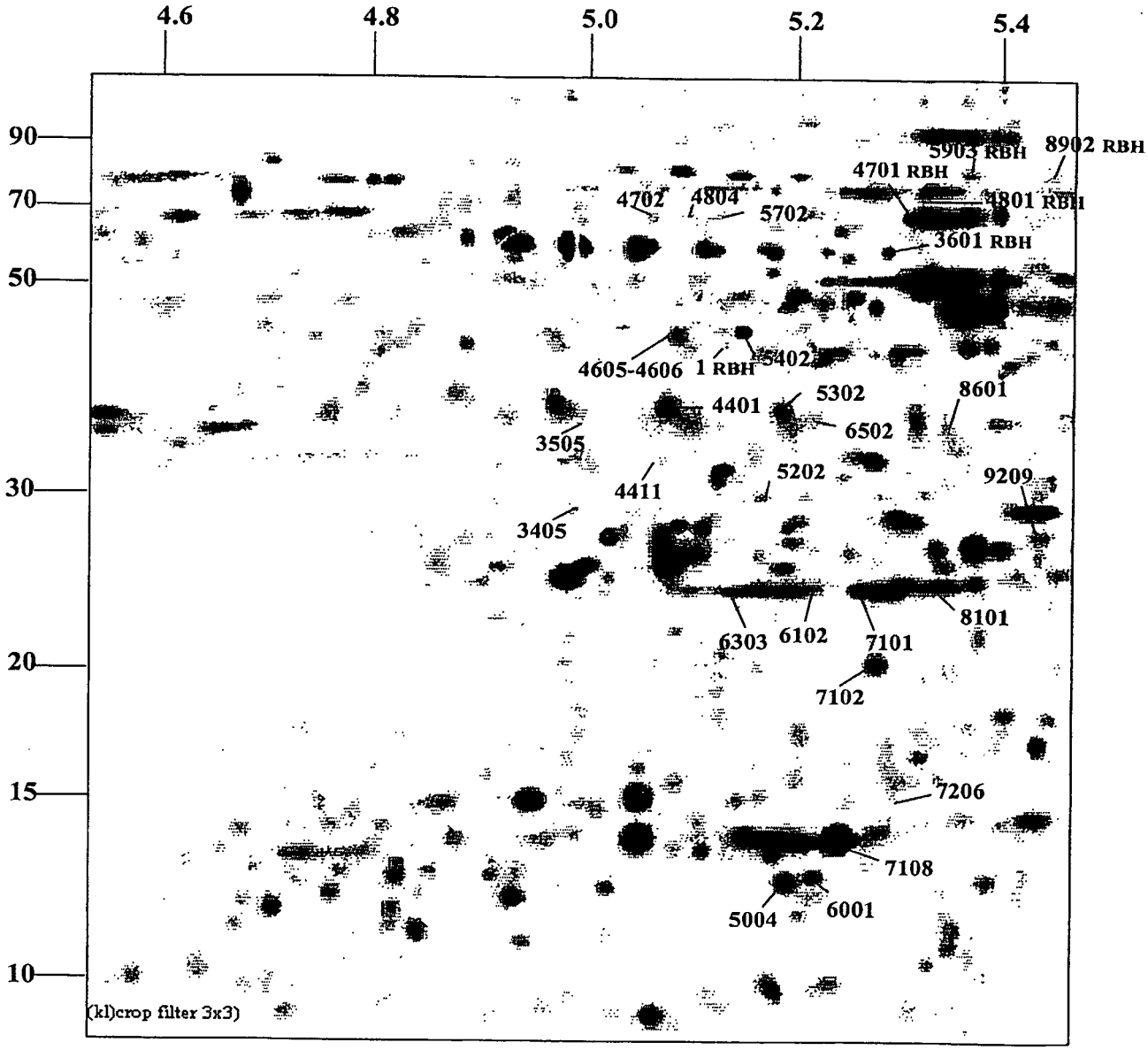
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Figure 1

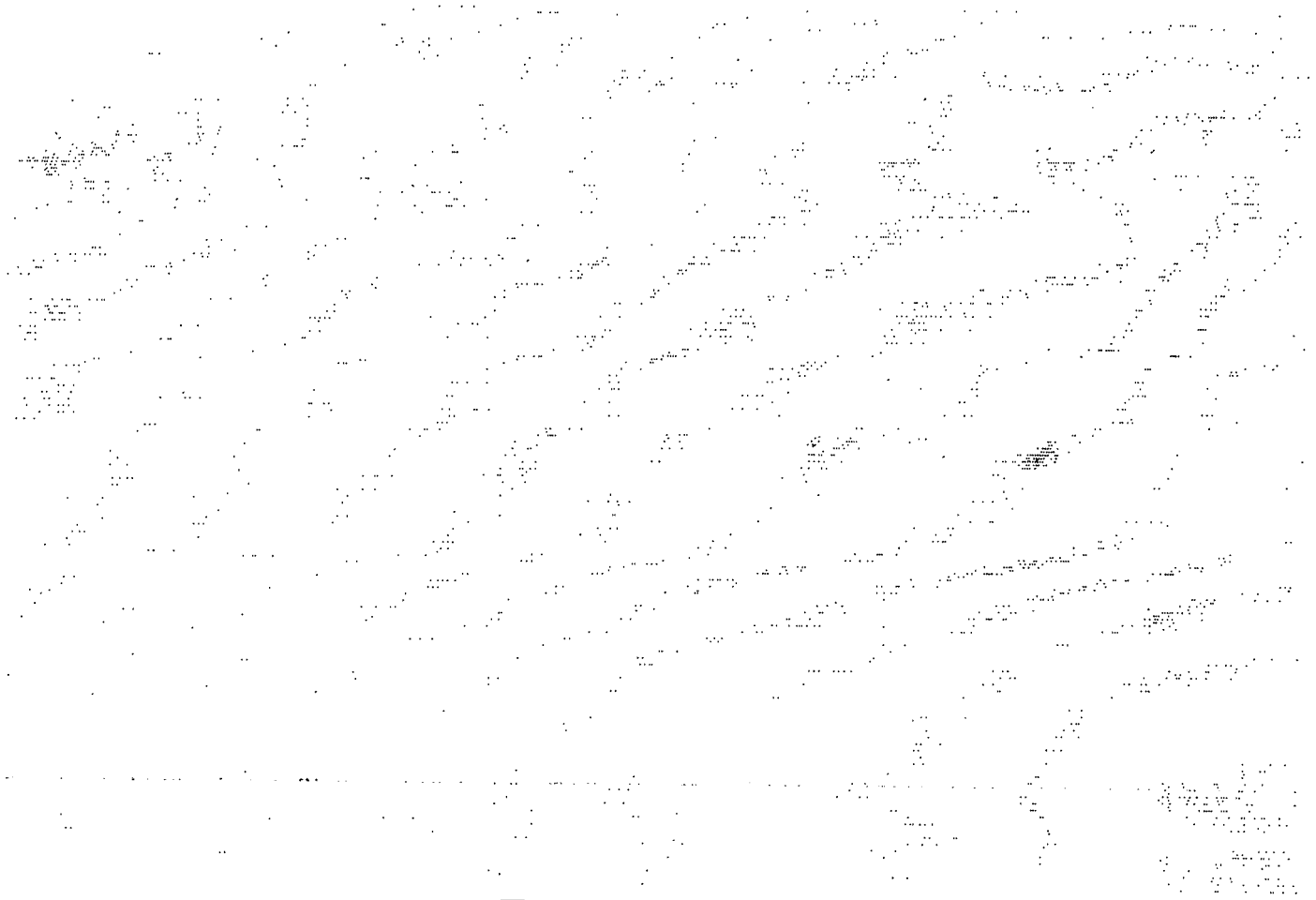

Figure 2

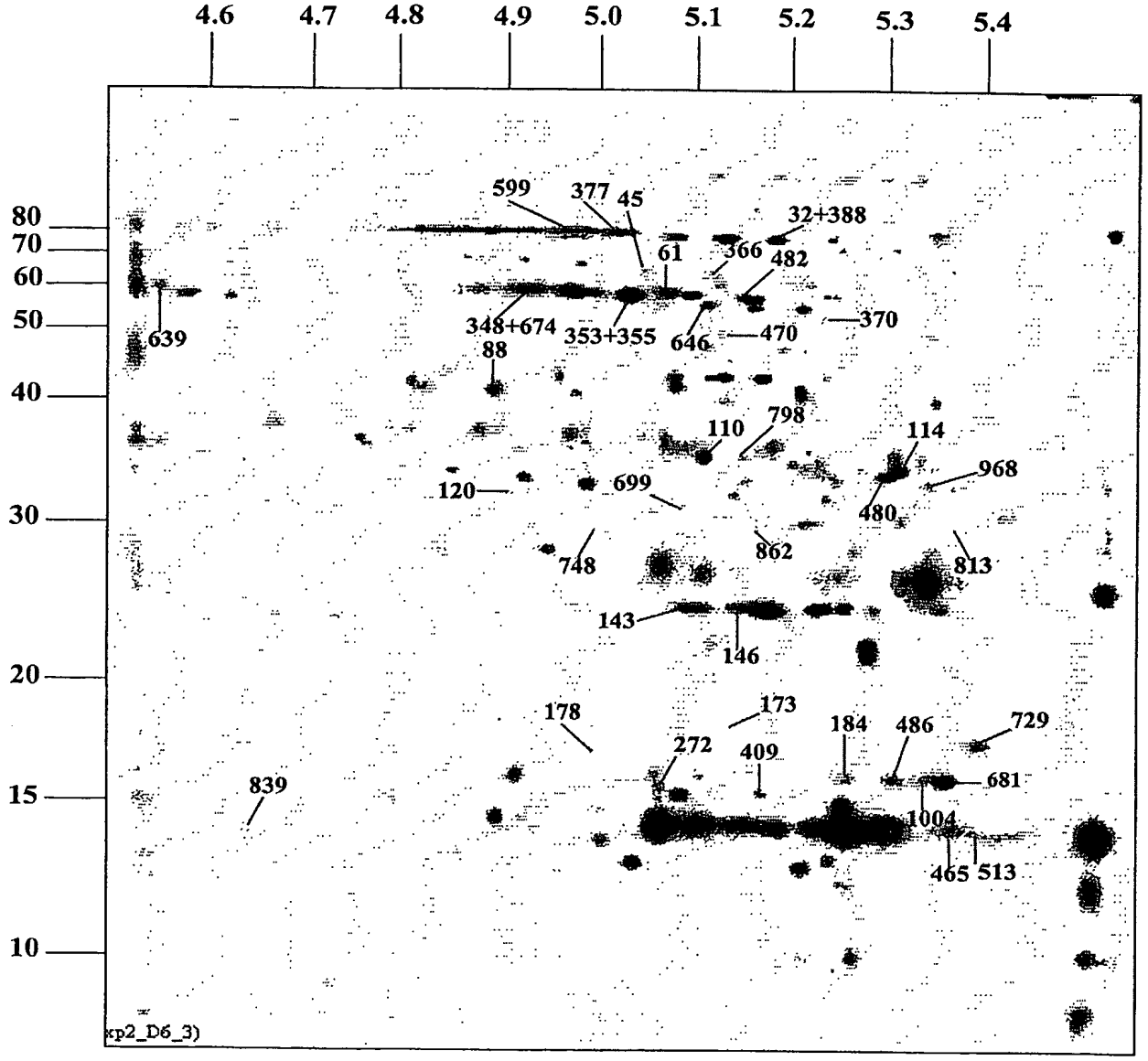
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Figure 3